BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

SANITARY DISTRICT OF DECATUR,	)	
	)	
Petitioner,	)	
	)	PCB 14-111
v.	)	(Variance - Water)
	)	
ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,	)	
	)	
	)	
Respondent.	)	

RECOMMENDATION OF THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

NOW COMES the Illinois Environmental Protection Agency ("Illinois EPA" or "Agency") by one of its attorneys, Sara Terranova, and files its Recommendation pursuant to 35 Ill. Adm. Code 104.216. The Illinois EPA recommends that the Illinois Pollution Control Board ("Board") **DENY** the Sanitary District of Decatur's ("District" or "Petitioner") request for a variance extension. In support of its Recommendation to DENY the variance extension, the Illinois EPA states as follows:

I. INTRODUCTION

1. On February 21, 2014, the District filed a Petition for Extension of Variance ("Petition") relating to their operation of a wastewater treatment plant ("Main Plant") in Decatur, Macon County, Illinois. The District requests to extend a 2010 variance ("Initial Variance") in which the Board granted from water quality standards for nickel at Section 302.208(e) of the Board's regulations (35 Ill. Adm. Code 302.208(e)) and from Section 304.105 of the Board's regulations (35 Ill. Adm. Code 304.105) as it applies to establishing water quality based effluent limits. *See* Initial Variance, PCB 09-125, January 7, 2010.
2. The District's National Pollutant Discharge Elimination System Permit ("NPDES") authorizes the

District to discharge into the Sangamon River. *See* NPDES permit No. IL 0028321, Exhibit A.

II. NOTICE

3. Pursuant to Section 104.214(a) of the Board's procedural rules, the Illinois EPA must provide notice of any petition for variance within 14 days after filing. *See* 35 Ill. Adm. Code 104.214(a). This Section provides that "the Agency must publish a single notice of such petition in a newspaper of general circulation in the county where the facility or pollution source is located." *See also* 415 ILCS 5/37(a)(2012). Section 104.214(b) requires the Illinois EPA to serve written notice of the petition on the County State's Attorney, the Chairman of the County Board, each member of the General Assembly from the legislative district in which the property is located, and any person in the county who has in writing requested notice of variance petitions. *See* 35 Ill. Adm. Code 104.214(b).
4. Consistent with 35 Ill. Adm. Code 104.214(a), the Illinois EPA published notice of the District's Petition for Extension of Variance in the Decatur *Herald & Review* on March 6, 2014. Consistent with Section 104.214(b), the Illinois EPA sent written notice of the petition to local officials on March 4, 2014.
5. On March, 21, 2014, the Agency received a comment letter ("2014 Letter") from Tinka G. Hyde, Director, Water Division, United State Environmental Protection Agency ("USEPA"). *See* USEPA March 21, 2014 Comment Letter, Exhibit B and C.
6. On April 2, 2014, the Agency received several questions, comments, and articles via email ("Emails") from a concerned citizen for consideration. *See* April 2, 2014 Emails from Emily Hood, Exhibit D.
7. Pursuant to the Board's procedural rules, "[w]ithin 21 days after the publication of notice, the Agency must file with the Board a certification of publication that states the date on which the notice was published and must attach a copy of the published notice." 35 Ill. Adm. Code 104.214(f).

8. Consistent with 35 Ill Adm. Code 104.214(f), on March 25, 2014, the Illinois EPA filed with the Board a certification of publication stating the date on which the notice was published and attached a copy of the published notice.

III. INVESTIGATION

9. The Illinois EPA is required to "promptly investigate such petition and consider the views of persons who might be adversely affected by the grant of the variance." *See* 415 ILCS 5/37(a) (2012) and 35 Ill. Adm. Code 104.216(a). Section 104.216(b)(1) requires the Agency to provide (i) a description of the efforts made to investigate the facts alleged and to ascertain the views of persons who might be affected, and (ii) a summary of the views so ascertained. *See* 35 Ill. Adm. Code 104.216(b)(1).
10. In preparing this Recommendation, the Illinois EPA consulted personnel within the Division of Water Pollution Control, including Rick Pinneo of the Permits Section and Brian Koch and Bob Mosher of the Water Quality Standards Section.
11. The Agency also carefully reviewed the 2014 Letter from Tinka G. Hyde of USEPA. *See* USEPA March 21, 2014 Comment Letter, Exhibit B. The 2014 Letter references USEPA's March 15, 2013 CITGO Variance Denial letter ("2013 Denial Letter") that disapproved Illinois' request for approval of a variance for CITGO Petroleum Corp. *Id* at 1. *See also* USEPA March 15, 2013 CITGO Variance Denial Letter, Exhibit C. The 2014 Letter states that as was explained in the 2013 Letter, under the Clean Water Act ("CWA") and USEPA's implementing regulations, a variance can only be approved by USEPA as a revision to water quality standards in accordance with section 303(c) of the CWA if, among other things, the State can demonstrate that the designated use for the water body at issue is not attainable for at least one of the reasons specified at 40 CFR 131.10(g). *Id* at 1.
12. The 2014 Letter further states this has been USEPA's long standing interpretation of the CWA and USEPA's implementing regulations and continues to be USEPA's interpretation. *Id* at 1. The 2014

Letter continues, explaining that for a variance to be approvable by USEPA under section 303(c) of the CWA and USEPA's implementing regulations, the Illinois EPA will be required to affirmatively demonstrate that attainment of the General Use designation for the Sangamon River is not feasible due to one of the reasons specified at 40 CFR 131.10(g). *Id* at 1. The 2014 Letter "urge[s] the Illinois EPA and the Board to carefully evaluate the District's variance request to determine whether this threshold has been met" and in doing so, should consider if all alternatives for reducing the discharge of nickel into the Sangamon River have been evaluated and demonstrated to be infeasible. *Id* at 1.

13. The 2014 Letter suggests considering "all alternatives for treating discharges from the District's wastewater treatment plant, all alternatives for reducing nickel in the wastewater from the ADM facility before it enters the District's sewer system such as treatment alternatives and process changes, and all alternatives for eliminating ADM's discharges into the District's sewer system such as piping ADM's discharges away from the sewer system to another receiving stream location where there might be more available dilution than currently exists in the portion of the Sangamon River into which the District discharges." *Id* at 1 and 2. Finally, the 2014 letter reiterates, as was explained in the 2013 Letter disapproving the CITGO variance, the feasibility threshold in section 131.10(g) is different from the "arbitrary and unreasonable hardship" threshold set forth in 415 ILCS 5/35(a). *Id* at 2.
14. The Agency also reviewed the questions, comments, and articles submitted via Email from Emily Hood. *See* April 2, 2014 Emails from Emily Hood, Exhibit D. Ms. Hood touched on many topics associated with the potential impacts to air and water quality due to point source contributions from ADM and Tate & Lyle, as well as from the District. *Id.*
15. The Emails raise air quality concerns regarding carbon monoxide complexing with nickel to form

nickel carbonyl and whether chronic air exposures were taken into consideration. *Id.*

16. As to water quality, the Emails put forth a number of questions, including whether "new nickel and zinc toxicology data" has been included in the variance extension, which seemingly is in reference to whether or not the District has developed site-specific water quality standards. *Id.*

17. Finally, the Emails pose whether the District is claiming that it is not required to meet Clean Water Act and Clean Air Act regulations due to fact that the majority of nickel and zinc emissions are from ADM and Tate & Lyle.

18. Additional and supplemental information provided by Ms. Hood is attached. *See* April 2, 2014 Emails from Emily Hood, Exhibit D.

IV. COST OF COMPLIANCE

19. Section 104.216(b)(5) of the Board's rules requires the Illinois EPA to estimate the cost that compliance would impose on the Petitioner and others. 35 Ill. Adm. Code 104.216(b)(5). Section 35(a) of the Environmental Protection Act ("Act") (415 ILCS 5/35(a) (2012)) requires the Board to determine if the petitioner has presented adequate proof that it would suffer an arbitrary or unreasonable hardship if required to immediately comply with the Board regulation at issue. *See* 415 ILCS 5/35(a)(2012).

20. As filed, the District's petition provides no discussion of the costs of immediate compliance. *See* 35 Ill. Adm. Code 104.210(b) and (d), 104.204(d). While the District has incorporated the entire PCB 09-125 record pursuant to 35 Ill. Adm. Code 104.210(d)(3), the Agency is unable to accurately ascertain what data from these files is still current and applicable, especially as the District has not made any such representations. The Agency is therefore unable to provide a current estimate of the costs that compliance would impose on the Petitioner and others.

V. IMPACT

21. When deciding to grant or deny a variance petition, the Board is required to balance the petitioner's hardship in complying with Board regulations against the impact that the requested variance will have on the environment. Monsanto Co. v. PCB, 67 Ill. 2d 276, 292, 367 N.E.2d 684, 691 (1977). Petitioner must establish that the hardship it would face from denial of its variance request would outweigh any injury to the public or the environment from granting the relief, and "[o]nly if the hardship outweighs the injury does the evidence rise to the level of an arbitrary or unreasonable hardship." Marathon Oil Co. v. EPA, 242 Ill. App. 3d 200, 206, 610 N.E. 2d 789, 793 (5th Dist. 1993).

22. Section 104.216(b)(6) of the Board's rules requires the Illinois EPA to estimate injury that the grant of the variance would impose on the public, including the effect that continued discharge of contaminants will have upon the environment. *See* 35 Ill. Adm. Code 104.216(b)(6).

23. As indicated in the Petition and Exhibit I of the Petition, the District, along with pretreatment facilities identified as significant sources of nickel and zinc loadings, has taken steps to reduce the concentrations of nickel and zinc received and discharged by Main Plant. Zinc influent and effluent reductions have been sufficiently effective that the District would be compliant with zinc permit limits. Therefore, the District is not seeking a variance extension relating to zinc. However, the District and ADM, the District's most significant industrial source of nickel, are still working towards attaining compliance with the chronic nickel limits. Two significant nickel reduction treatment processes have been installed at ADM and a third (a precipitation and filtration treatment system for ADM's Polyol manufacturing process) is presently being installed.

24. Despite the past and ongoing nickel reductions, complete attainment of the chronic nickel water quality standard is not presently achievable by the District. Thus, a potential for environmental

impacts to the District's receiving water, Segment E-09 of the Sangamon River, exists. Under drought conditions when the District's receiving water contains low stream flow, excess concentrations of nickel from the District occasionally lead to in-stream excursions of the chronic nickel water quality standard. However, despite these excursions (See Petitioner's Exhibit I), District effluent has not had an appreciable effect on aquatic life in the receiving water, as the receiving water has been and continues to be fully supportive of aquatic life use as determined by Illinois EPA and summarized in *Final Illinois IR for 2014*.

25. Additionally, Eastern Illinois University ("EIU") biotic assessments, performed in 1998 and annually from 2001-2014, conducted upstream and downstream of the District's discharge point have not shown an appreciable environmental impact from the District's effluent. In fact, according to these EIU studies, slight improvements in biotic communities have been observed downstream of the District. However, this may be more so attributed to the consistent flow existing downstream of the discharge point, rather than the quality of the effluent being discharged.

26. While Illinois EPA is concerned that nickel concentrations in the District's effluent may be harmful due to exceedances of the chronic nickel water quality standard, Illinois EPA is also cognizant that the existing hardness-based chronic nickel standard applicable to the receiving water may not be entirely representative of nickel toxicity due to site-specific water quality. Based on the physical and chemical characteristics of the receiving water, a site-specific chronic nickel water quality standard using a multitude of parameters influencing nickel toxicity (e.g. pH, hardness, dissolved organic carbon) may be more appropriate than the General Use standard based solely on hardness. Therefore, the District, along with oversight from Illinois EPA and USEPA, is currently working towards the development of site specific nickel water quality standards for its receiving water. Once developed, a re-evaluation of the environmental impacts from the District's discharge may be necessary.

VI. COMPLIANCE PLAN

27. Pursuant to section 104.204(f), the Petitioner is required to present a detailed description of the compliance plan. *See* 35 Ill. Adm. Code 104.204(f). The District does provide a plan with suggested conditions. However, the District provides no detailed description of how the plan will bring them into compliance. *See* Petition For Extension of Variance at 12 and 13.

28. Suggested Condition f. in the District's compliance plan provides the District will "achieve compliance with the District's NPDES permit effluent limits for nickel" by July 1, 2015. *Id.* at13. The District provides no details on, or explanation as to how that is possible given that the appropriate research required for the development of a site specific nickel water quality standard for the District's receiving water is still on-going. One possibility is that the District will come into compliance with the existing permit limits by implementing the third nickel reduction treatment process at ADM. However, the District notes that "reducing nickel concentration reductions in the District's influent will not, by itself, allow the District to achieve compliance with its current NPDES discharge limit for Nickel." *Id* at 11. Therefore, the Agency is unable to ascertain how compliance with the District's NPDES permit effluent limits for nickel by July 1, 2015 is possible.

VII. CONSISTENCY WITH FEDERAL LAW

29. Section 104.216(b)(7) of the Board's rules requires the Agency to provide an analysis of applicable federal laws and regulations as well as an opinion concerning the consistency of the petition with such federal laws and regulations. See 35 Ill. Adm. Code 104.216(b)(7).

ANALYSIS OF APPLICABLE FEDERAL LAW AND REGULATIONS

Designated Uses and Water Quality Criteria

30. Section 101(a)(2) of the Clean Water Act ("CWA") states the national interim goal of achieving by July 1, 1983, "water quality which provides for the protection and propagation of fish, shellfish, and

wildlife and provides for recreation in and on the water" (hereafter collectively referred to as "the uses specified in section 101(a)(2)"), wherever attainable. Section 303 of the CWA requires states to adopt water quality standards for waters of the United States within their respective jurisdictions. Section 303(c) of the CWA requires, among other things, that state water quality standards include the designated use or uses to be made of the waters and water quality criteria based upon such uses.[1]

31. USEPA's regulations at 40 CFR Part 131 interpret and implement sections 101(a)(2) and 303(c) of the CWA through a requirement that water quality standards include the uses specified in section 101(a)(2) of the CWA, unless those uses have been shown to be unattainable, in which case a state can adopt subcategories of the uses specified in section 101(a)(2) which require less stringent criteria. *See* 40 CFR 131.5(a)(4), 131.6(a), and 131.10(j), and 131.20(a); *see also* Idaho Mining Association v. Browner,.90 F.Supp. 2d 1078, 1092 (D. Id. 2000); 68 Fed. Reg. 40428, 40430-31 (July 27, 2003).

32. Federal regulations regarding the designation of uses are found in 40 CFR 131.10.[2] Section 131.10(g) provides that, once a state designates the uses specified in section 101(a)(2) of the CWA or subcategories thereof for a specific water body, the state can only remove the designated use if, among other things, the state can demonstrate that attaining the designated use is not feasible for at

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[1] Section 303(c)(2)(A) of the CWA requires that water quality standards "protect the public health or welfare, enhance the quality of water and serve the purposes" of the CWA. USEPA's regulations at 40 CFR 131.2 explain that:

> "Serve the purposes of the Act" (as defined in sections 101(a)(2) and 303(c) of the Act) means that water quality standards should, wherever attainable, provide water quality for the protection and propagation of fish, shellfish and wildlife and for recreation in and on the water and take into consideration their use and value of public water supplies, propagation of fish, shellfish, and wildlife, recreation in and on the water, and agricultural, industrial, and other purposes including navigation.

[2] When a state adopts designated uses that include the uses specified in section 101(a)(2) of the CWA or subcategories thereof, the state must also adopt "water quality criteria that protect the designated use." 40 CFR 131.11 (a). "Such criteria must be based on sound scientific rationale and must contain sufficient parameters or constituents to protect the designated use." *Id.*

least one of the six reasons set at 40 CFR 131.10(g)[3].

33. Unlike with designated uses, nothing in the CWA or USEPA's regulations allows states to relax or modify criteria, based on concepts of attainability, to levels that are not protective of the designated use. Instead, if criteria are not attainable, the CWA and USEPA's regulations allow states to (1) remove the current designated use after demonstrating, among other things, that attaining the current designated use is not feasible for one of the 40 CFR 131.10(g) reasons, and replace it with a subcategory of use and, then, (2) adopt new, potentially less stringent, criteria necessary to protect the new designated use.

Variances

34. USEPA provides it has long recognized[4] it could also approve a state decision to limit the applicability of the use removal to only a single discharger, while continuing to apply the previous use designation and criteria to other dischargers. Such a state decision, which is often referred to as a

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[3]40 CFR 101.10(g): States may remove a designated use which is *not* an existing use, as defined in §131.3, or establish sub-categories of a use if the State can demonstrate that attaining the designated use is not feasible because:
(1) Naturally occurring pollutant concentrations prevent the attainment of the use; or
(2) Natural, ephemeral, intermittent or low flow conditions or water levels prevent the attainment of the use, unless these conditions may be compensated for by the discharge of sufficient volume of effluent discharges without violating State water conservation requirements to enable uses to be met; or
(3) Human caused conditions or sources of pollution prevent the attainment of the use and cannot be remedied or would cause more environmental damage to correct than to leave in place; or
(4) Dams, diversions or other types of hydrologic modifications preclude the attainment of the use, and it is not feasible to restore the water body to its original condition or to operate such modification in a way that would result in the attainment of the use; or
(5) Physical conditions related to the natural features of the water body, such as the lack of a proper substrate, cover, flow, depth, pools, riffles, and the like, unrelated to water quality, preclude attainment of aquatic life protection uses; or
(6) Controls more stringent than those required by sections 301(b) and 306 of the Act would result in substantial and widespread economic and social impact.

[4] USEPA March 21, 2014 Comment Letter, Exhibit B., USEPA March 15, 2013 CITGO Variance Denial Letter, Exhibit C. Decision of the General Counsel No. 44, June 22, 1976., Decision of the General Counsel No. 58, March 29, 1977 (published, in part, at 44 F.R. 39508 (July 6, 1979))., EPA's definition of a WQS variance to the Regional WQS Coordinators, July 3, 1979., Director of the Office of Water Regulations and Standards, responding to questions raised on WQS variances, issued a reinterpretation of the factors that could be considered when granting variances, March 1985., Water Quality Standards Handbook - Chapter 5: General Policies pp.: 5-1–5-12.

"variance," can be approved as being consistent with the requirements of the CWA and 40 CFR Part 131. This is because the state's action in limiting the applicability of an otherwise approvable use removal to a single discharger and to a single pollutant is environmentally preferable and would be more stringent than a full use removal. States have the right to establish more stringent standards under section 510 of the CWA. See 58 FR 20802, 20921-22 (April16, 1993).

USEPA Proposed Rule

35. On September 4, 2013, USEPA proposed clarification revisions to USEPA's water quality standards regulations. *See* Water Quality Standard Regulatory Clarifications,78 Fed Reg.54518 (Sept 4, 2013).

36. The proposed revision adds Section 131.14 to establish regulatory guidelines for Water Quality Standard ("WQS") variances and WQS variance renewals, including that a WQS variance submission must specify:

    a. the pollutant(s), the permittee(s), and/or the waterbody or water by segments to which the WQS variance applies;
    b. numeric interim requirements that apply during the WQS variance for CWA section 402 NPDES permitting and section 401 certification;
    c. an expiration date not to exceed 10 years; and
    d. a section 131.10(g) factor to justify why and for how long a WQS variance is necessary.

37. A WQS variance will be defined as "a time-limited use and criterion for a specified pollutant(s), permittee(s), and/or waterbody or waterbody segment(s) that reflect the highest attainable condition during the specified time period." *See* EPA 820-F-13-026, Summary of Water Quality Standards Regulatory Clarifications Proposed Rule, August 2013.

38. In the 2014 Letter, USEPA informed the Agency, "a variance can only be approved by the USEPA as a revision to water quality standards in accordance with section 303(c) of the CWA if, among other things, the State can demonstrate that the designated use for the water body at issue is not attainable for at least one of the reasons specified in 40 CFR 131.10(g)." *See* USEPA March 21,

2014 Comment Letter, Exhibit B. USEPA went on to say, "this continues to be USEPA's interpretation and nothing in the Federal Register notice or in the USEPA's proposed revisions to its water quality regulations changes that longstanding interpretation." *Id.*

Water Quality Submission Requirements and USEPA Review Authority

39. Pursuant to 40 CFR 131.6, states must submit, among other things, the following to the USEPA for review when they adopt new or revised designated uses and criteria:

    a. Use designations consistent with the provisions of section 101(a)(2) and 303(c)(2)of the CWA.
    b. Methods used and analyses conducted to support water quality standards revisions.
    c. Water quality criteria to protect the designated uses.
    ....
    f. General information which will aid the Agency in determining the adequacy of the scientific basis of the standards which do not include the uses specified in section 101(a)(2) of the CWA as well as information on general policies applicable to State standards which may affect their application and implementation.

40. Pursuant to 40 CFR 131.5 5(a), in reviewing new or revised use designations and criteria, the USEPA must determine, among other things:

    1. Whether the State has adopted water uses which are consistent with the requirements of the Clean Water Act;
    2. Whether the State has adopted criteria that protect the designated uses;
    ...
    4. Whether the State standards which do not include the uses specified in section 101 (a)(2) of the Act are based upon appropriate technical and scientific data and analyses; and
    5. Whether the State submission meets the requirements included in § 131.6 of this part.

41. Pursuant to 40 CFR 131.21(c)(2), new or revised water quality standards that are adopted by states do not become applicable water quality standards for purposes of the CWA until after they have been submitted to and approved by USEPA in accordance with section 303(c) of the CWA.

ILLINOIS EPA OPINION CONCERNING THE CONSISTENCY OF THE PETITION WITH FEDREAL LAW AND REGULATIONS

42. Under USEPA's regulations and interpretations, a state can only remove a designated use specified in section 101(a)(2) of the CWA, or a subcategory thereof, if, among other things, the state demonstrates that it is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g). *See* 2013 Denial Letter. USEPA holds that the CWA and federal regulations do not allow states to remove designated uses or modify criteria simply because a state believes that such standards "would impose an arbitrary or unreasonable hardship." *Id.*

43. Petitioner makes no demonstration that it is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g).

44. As was set forth in the 2013 Denial Letter, and reiterated in the 2014 Letter, USEPA will not approve a variance request that does not affirmatively demonstrate that attainment of the designated use is not feasible for one of the reasons specified at 40 CFR 131.10(g). Without such a demonstration, a variance granted by the Board will be disapproved by the USEPA pursuant to its stated regulations and longstanding policy and interpretations. Therefore, until the District demonstrates that is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g), the Agency concludes the Petition is inconsistent with Federal Law and Regulations.

VIII. PERMITS AND ENFORCMENT ACTION

45. Section 104.214(b)(8) of the Board's rules requires the Illinois EPA to discuss in its recommendation the status of any permits or pending permit applications that are associated with or affected by the requested variance. 35 Ill. Adm. Code 104.216(b)(8).

46. The District's NPDES Permit was issued April 20, 2007 and became effective July 1, 2007. This permit has an expiration date of June 30, 2012. This permit was modified July 1, 2009. A

modification request was received by the Agency June 20, 2010 and a draft of the modification was sent to 15-day notice April 12, 2011. The permit was then sent to 30-day notice on May 20, 2011. The Agency received comments from Prairie River Network as well as from the District. A public hearing was not held for this modification request. The Agency received a renewal application on December 27, 2011.

47. In addition to the Initial Variance, the Board granted Petitioner a Site Specific Rule exempting the District from certain biochemical oxygen demand and suspended solids discharge limits. This Site Specific Rule can be found at Section 304.212 of Title 35 of the Board regulations. *See* 35 Ill. Adm. Code 304.212.

48. The Illinois EPA is required by Section 104.216(b)(4) to inform the Board of any past or pending enforcement actions against the Petitioner. *See* 35 Ill. Adm. Code 104.216(b)(4).

49. The District was issued four Violation Notices for Overflows from Sanitary Sewers since the Initial Variance. Violation Notice W-2011-50444, issued November 2, 2011, cited a sanitary sewer overflow. Following the Violation Notice the District returned to compliance. Violation Notice W-2011-50421, issued November 2, 2011, cited a sanitary sewer overflow. The Agency accepted the District's response on December 22, 2011. Violation Notice W-2012-50173, issued on July 9, 2012, cited a sanitary sewer overflow. The Agency accepted the District's response on August 29, 2012. Violation Notice W-2013-50013, issued on February 13, 2013, cited a sanitary sewer overflow. The Agency accepted the District's response on April 23, 2013.

50. USEPA has an on-going case with the District for sanitary sewer overflows. Therefore, the Agency is currently sending all new (since April 2013) sanitary sewer overflow violations to USEPA.

51. The District was issued three Violation Notices for Overflows from Sanitary Sewers in 2009. Violation Notice W-2009-00181, issued on July 2, 2009, cited a sanitary sewer overflow that

occurred on May 24, 2009. Violation Notice W-2009-00188, issued July 2, 2009, cited a sanitary sewer overflow that occurred on May 29, 2009. Violation Notice W-2009-00189, also issued July 2, 2009, cited a sanitary sewer overflow that occurred on June 1, 2009.

52. Finally, the District has been the respondent to at least four Illinois EPA enforcement actions that occurred more than 20 years ago:

    a. a case filed in U.S. District Court on December 17, 1982 that resulted in the District paying a civil penalty of $1000;

    b. case number PCB 1977-238 was a Water enforcement case against both the City of Decatur and Decatur Sanitary District that involved a fishkill resulting from discharges from the combined sewer and wastewater treatment plant;

    c. case number PCB 1977-157 was a mixed media enforcement case against the District, A.E. Staley Manufacturing Company and ADM that involved among other issues, violations of the dissolved oxygen limits set in the District's NPDES permit; and

    d. case number PCB 1976-181 was an Air enforcement case (listed as a Land enforcement case) on the Board's website at http://www.ipcb.state.il.us/COOL/external/CaseView.aspx?referer=results&case=10015 against the District that involved excessive odors at the sewage treatment plant.

IX. RECOMMENDATION

53. Given that the District needs to perform additional Biotic Ligand Model ("BLM") work and ADM has yet to implement new pretreatment technology to reduce nickel, the District is clearly in need of additional time by which to achieve compliance with the applicable nickel water quality

standard. The Agency believes that additional time of one year would be sufficient to complete the appropriate BLM research required to validate the resulting site-specific nickel standard and file a petition with the Board. This additional time would also allow the District to conduct the confirmatory Water Effects Ratio ("WER") testing, should it choose to do so. The request to seek this additional time through a variance however must comply with state as well as federal requirements. As discussed in detail under the Consistency With Federal Law heading, the District has failed to comply with the mandatory federal requirements. The District maintains that there is no valid current applicable federal law or regulation that precludes the Board's granting the District's variance extension request. However, under USEPA's regulations and interpretations, the District can only remove a designated use specified in section 101(a)(2) of the CWA, or a subcategory thereof, if, among other things, the District demonstrates that it is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g).

54. Based on the totality of the factors stated above, the Illinois EPA recommends that the Board DENY the Petitioner's request for an extension of variance from water quality standards for nickel at Section 302.208(e) of the Board's regulations (35 Ill. Adm. Code 302.208(e)) and from Section 304.105 of the Board's regulations (35 Ill. Adm. Code 304.105) as it applies to establishing water quality based effluent limits.

Wherefore, for the reasons stated above, the Illinois EPA recommends that the Board **DENY** the extension of variance requested by the Sanitary District of Decatur.

Respectfully submitted,

Dated: April 7, 2014
1021 North Grand Avenue East
PO Box 19276
Springfield IL 62794-9276
217-782-5544

By: Sara Terranova
Sara Terranova
Assistant Counsel
Illinois EPA

Exhibit A

NPDES Permit No. IL0028321

Illinois Environmental Protection Agency

Division of Water Pollution Control

1021 North Grand Avenue East

Post Office Box 19276

Springfield, Illinois 62794-9276

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM

Modified (NPDES) Permit

Expiration Date: June 30, 2012

Issue Date: April 20, 2007
Effective Date: July 1, 2007
Modification Date: July 1, 2009

Name and Address of Permittee:

Sanitary District of Decatur
501 Dipper Lane
Decatur, Illinois 62522

Facility Name and Address:

Sanitary District of Decatur Main STP
501 Dipper Lane
Decatur, Illinois
(Macon County)

Receiving Waters: Sangamon River

In compliance with the provisions of the Illinois Environmental Protection Act, Title 35 of the Ill. Adm. Code, Subtitle C, Chapter I, and the Clean Water Act (CWA), the above-named Permittee is hereby authorized to discharge at the above location to the above-named receiving stream in accordance with the standard conditions and attachments herein.

Permittee is not authorized to discharge after the above expiration date. In order to receive authorization to discharge beyond the expiration date, the Permittee shall submit the proper application as required by the Illinois Environmental Protection Agency (IEPA) not later than 180 days prior to the expiration date.

Alan Keller by REP

Alan Keller, P.E.
Manager, Permit Section
Division of Water Pollution Control

SAK:REP:06120503.bah

Modification Date: July 1, 2009

NPDES Permit No. IL0028321

Effluent Limitations, Monitoring, and Reporting

FINAL

Discharge Number(s) and Name(s): 001 STP Outfall

Load limits computed based on a design average flow (DAF) of 41.0 MGD (design maximum flow (DMF) of 125.0 MGD).

Excess flow facilities (if applicable) shall not be utilized until the main treatment facility is receiving its maximum practical flow.

From the modification date of this Permit until the expiration date, the effluent of the above discharge(s) shall be monitored and limited at all times as follows:

	LOAD LIMITS lbs/day DAF (DMF)*			CONCENTRATION LIMITS MG/L				
Parameter	Monthly Average	Weekly Average	Daily Maximum	Monthly Average	Weekly Average	Daily Maximum	Sample Frequency	Sample Type
Flow (MGD)							Continuous	
$CBOD_5$**	6,839 (20,850)	13,678 (41,700)		20	40		2 days/week	Composite
Suspended Solids	8,549 (26,063)	15, 387 (46,913)		25	45		2 days/week	Composite
Dissolved Oxygen	Shall not be less than 6 mg/L						2 days/week	Grab
pH	Shall be in the range of 6 to 9 Standard Units						2 days/week	Grab
Fecal Coliform***	Daily Maximum shall not exceed 400 per 100 mL (May through October)						2 days/week	Grab
Chlorine Residual***						0.05	2 days/week	Grab
Ammonia Nitrogen as (N)								
March-May/Sept.-Oct.	513 (1,564)		1,026 (3,128)	1.5		3.0	2 days/week	Composite
June-August	445 (1,355)		1,026 (3,128)	1.3		3.0	2 days/week	Composite
Nov.-Feb.	513 (1,564)		1,026 (3,128)	1.5		3.0	2 days/week	Composite
Zinc ****	26 (78)		142 (434)	0.075		0.416	5 days/week	Composite
Nickel ****	5.1 (16)			0.015			5 days/week	Composite

*Load limits based on design maximum flow shall apply only when flow exceeds design average flow.
**Carbonaceous BOD_5 ($CBOD_5$) testing shall be in accordance with 40 CFR 136.
***See Special Condition 7.
****See Special Condition 17.

Flow shall be reported on the Discharge Monitoring Report (DMR) as monthly average and daily maximum.

Fecal Coliform shall be reported on the DMR as daily maximum.

pH shall be reported on the DMR as a minimum and a maximum.

Chlorine Residual shall be reported on DMR as daily maximum.

Dissolved oxygen shall be reported on DMR as minimum.

 Modification Date: July 1, 2009

NPDES Permit No. IL0028321

Effluent Limitations, Monitoring, and Reporting

FINAL

Discharge Number(s) and Name(s): 003 Oakland Avenue Treated Combined Sewage Outfall
004 South Edward Street Treated Combined Sewage Outfall
007 McKinley Avenue Treated Combined Sewage Outfall
008 Seventh Ward Treated Combined Sewage Outfall

These flow facilities shall not be utilized until the main treatment facility is receiving its maximum practical flow.

From the modification date of this Permit until the expiration date, the effluent of the above discharge(s) shall be monitored and limited at all times as follows:

Parameter		CONCENTRATION LIMITS mg/L Monthly Average	Sample Frequency	Sample Type
Total Flow (MG)	See Below		Daily When Discharging	Continuous
BOD_5			Daily When Discharging	Grab
Suspended Solids			Daily When Discharging	Grab
pH	Shall be in the range of 6 to 9 Standard Units		Daily When Discharging	Grab

Total flow in million gallons shall be reported on the Discharge Monitoring Report (DMR) in the quantity maximum column.

Report the number of days of discharge in the comments section of the DMR.

pH shall be reported on the DMR as a minimum and a maximum.

BOD_5 and Suspended Solids shall be reported on the DMR as a monthly average concentration.

Modification Date: July 1, 2009

NPDES Permit No. IL0028321

Influent Monitoring, and Reporting

The influent to the plant shall be monitored as follows:

Parameter	Sample Frequency	Sample Type
Flow (MGD)	Continuous	*RIT
BOD_5	2 days/week	Composite
Suspended Solids	2 days/week	Composite

Influent samples shall be taken at a point representative of the influent.

Flow (MGD) shall be reported on the Discharge Monitoring Report (DMR) as monthly average and daily maximum.

BOD_5 and Suspended Solids shall be reported on the DMR as a monthly average concentration.

*Recording, Indicating, Totalizing.

NPDES Permit No. IL0028321

Special Conditions

SPECIAL CONDITION 1. This Permit may be modified to include different final effluent limitations or requirements which are consistent with applicable laws, regulations, or judicial orders. The IEPA will public notice the permit modification.

SPECIAL CONDITION 2. The use or operation of this facility shall be by or under the supervision of a Certified Class 1 operator.

SPECIAL CONDITION 3. The IEPA may request in writing submittal of operational information in a specified form and at a required frequency at any time during the effective period of this Permit.

SPECIAL CONDITION 4. The IEPA may request more frequent monitoring by permit modification pursuant to 40 CFR § 122.63 and Without Public Notice in the event of operational, maintenance or other problems resulting in possible effluent deterioration.

SPECIAL CONDITION 5. The effluent, alone or in combination with other sources, shall not cause a violation of any applicable water quality standard outlined in 35 Ill. Adm. Code 302.

SPECIAL CONDITION 6. Samples taken in compliance with the effluent monitoring requirements shall be taken at a point representative of the discharge, but prior to entry into the receiving stream.

SPECIAL CONDITION 7. Fecal Coliform limits for Discharge Number 001 are effective May thru October. Sampling of Fecal Coliform is only required during this time period.

The total residual chlorine limit is applicable at all times. If the Permittee is chlorinating for any purpose during the months of November through April, sampling is required on a daily grab basis. Sampling frequency for the months of May through October shall be as indicated on effluent limitations, monitoring and reporting page of this Permit.

SPECIAL CONDITION 8.

A. Publicly Owned Treatment Works (POTW) Pretreatment Program General Provisions

1. The Permittee shall implement and enforce its approved Pretreatment Program which was approved on September 3, 1985 and all approved subsequent modifications thereto. The Permittee shall maintain legal authority adequate to fully implement the Pretreatment Program in compliance with Federal (40 CFR 403), State, and local laws. The Permittee shall:

   a. Carry out independent inspection and monitoring procedures at least once per year, which will determine whether each significant industrial user (SIU) is in compliance with applicable pretreatment standards;

   b. Perform an evaluation, at least once every two (2) years, to determine whether each SIU needs a slug control plan. If needed, the SIU slug control plan shall include the items specified in 40 CFR § 403.8 (f)(2)(v);

   c. Update its inventory of Industrial Users (IUs) at least annually and as needed to ensure that all SIUs are properly identified, characterized, and categorized;

   d. Receive and review self monitoring and other IU reports to determine compliance with all pretreatment standards and requirements, and obtain appropriate remedies for noncompliance by any IU with any pretreatment standard and/or requirement;

   e. Investigate instances of noncompliance, collect and analyze samples, and compile other information with sufficient care as to produce evidence admissible in enforcement proceedings, including judicial action;

   f. Require development, as necessary, of compliance schedules by each industrial user for the installation of control technologies to meet applicable pretreatment standards; and,

   g. Maintain an adequate revenue structure for continued operation of the Pretreatment Program.

2. The Permittee shall issue/reissue permits or equivalent control mechanisms to all SIUs prior to expiration of existing permits or prior to commencement of discharge in the case of new discharges. The permits at a minimum shall include the elements listed in 40 CFR § 403.8(f)(1)(iii).

3. The Permittee shall develop, maintain, and enforce, as necessary, local limits to implement the prohibitions in 40 CFR § 403.5 which prohibit the introduction of specific pollutants to the waste treatment system from any source of nondomestic discharge.

Special Conditions

4. In addition to the general limitations expressed in Paragraph 3 above, applicable pretreatment standards must be met by all industrial users of the POTW. These limitations include specific standards for certain industrial categories as determined by Section 307(b) and (c) of the Clean Water Act, State limits, or local limits, whichever are more stringent.

5. The USEPA and IEPA individually retain the right to take legal action against any industrial user and/or the POTW for those cases where an industrial user has failed to meet an applicable pretreatment standard by the deadline date regardless of whether or not such failure has resulted in a permit violation.

6. The Permittee shall establish agreements with all contributing jurisdictions, as necessary, to enable it to fulfill its requirements with respect to all IUs discharging to its system.

7. Unless already completed, the Permittee shall within six (6) months of the effective date of this Permit submit to USEPA and IEPA a proposal to modify and update its approved Pretreatment Program to incorporate Federal revisions to the general pretreatment regulations. The proposal shall include all changes to the approved program and the sewer use ordinance which are necessary to incorporate the regulations commonly referred to as PIRT and DSS, which were effective November 16, 1988 and August 23, 1990, respectively. This includes the development of an Enforcement Response Plan (ERP) and a technical re-evaluation of the Permittee's local limits.

8. The Permittee's Pretreatment Program has been modified to incorporate a Pretreatment Program Amendment approved on February 6, 1995. The amendment became effective on the date of approval and is a fully enforceable provision of your Pretreatment Program.

   Modifications of your Pretreatment Program shall be submitted in accordance with 40 CFR § 403.18, which established conditions for substantial and nonsubstantial modifications.

B. Reporting and Records Requirements

1. The Permittee shall provide an annual report briefly describing the permittee's pretreatment program activities over the previous calendar year. Permittees who operate multiple plants may provide a single report providing all plant-specific reporting requirements are met. Such report shall be submitted no later than April 28 of each year, and shall be in the format set forth in IEPA's POTW Pretreatment Report Package which contains information regarding:

   a. An updated listing of the Permittee's industrial users.

   b. A descriptive summary of the compliance activities including numbers of any major enforcement actions, (i.e., administrative orders, penalties, civil actions, etc.), and the outcome of those actions. This includes an assessment of the compliance status of the Permittee's industrial users and the effectiveness of the Permittee's Pretreatment Program in meeting its needs and objectives.

   c. A description of all substantive changes made to the Permittee's Pretreatment Program. Changes which are "substantial modifications" as described in 40 CFR § 403.18(c) must receive prior approval from the Approval Authority.

   d. Results of sampling and analysis of POTW influent, effluent, and sludge.

   e. A summary of the findings from the priority pollutants sampling. As sufficient data becomes available the IEPA may modify this Permit to incorporate additional requirements relating to the evaluation, establishment, and enforcement of local limits for organic pollutants. Any permit modification is subject to formal due process procedures pursuant to State and Federal law and regulation. Upon a determination that an organic pollutant is present that causes interference or pass through, the Permittee shall establish local limits as required by 40 CFR § 403.5(c).

2. The Permittee shall maintain all pretreatment data and records for a minimum of three (3) years. This period shall be extended during the course of unresolved litigation or when requested by the IEPA or the Regional Administrator of USEPA. Records shall be available to USEPA and the IEPA upon request.

Special Conditions

3. The Permittee shall establish public participation requirements of 40 CFR 25 in implementation of its Pretreatment Program. The Permittee shall at least annually, publish the names of all IU's which were in significant noncompliance (SNC), as defined by 40 CFR § 403.8(f)(2)(vii), in the largest daily paper in the municipality in which the POTW is located or based on any more restrictive definition of SNC that the POTW may be using.

4. The Permittee shall provide written notification to the Deputy Counsel for the Division of Water Pollution Control, IEPA, 1021 North Grand Avenue East, P.O. Box 19276, Springfield, Illinois 62794-9276 within five (5) days of receiving notice that any Industrial User of its sewage treatment plant is appealing to the Circuit Court any condition imposed by the Permittee in any permit issued to the Industrial User by Permittee. A copy of the Industrial User's appeal and all other pleadings filed by all parties shall be mailed to the Deputy Counsel within five (5) days of the pleadings being filed in Circuit Court.

C. Monitoring Requirements

1. The Permittee shall monitor its influent, effluent and sludge and report concentrations of the following parameters on monitoring report forms provided by the IEPA and include them in its annual report. Samples shall be taken at quarterly (four times per year) intervals at the indicated reporting limit or better and consist of a 24-hour composite unless otherwise specified below. Sludge samples shall be taken of final sludge and consist of a grab sample reported on a dry weight basis.

STORET CODE	PARAMETER	Minimum reporting limit
01097	Antimony	0.07 mg/L
01002	Arsenic	0.05 mg/L
01007	Barium	0.5 mg/L
01012	Beryllium	0.005 mg/L
01027	Cadmium	0.001 mg/L
01032	Chromium (hex - grab not to exceed 24 hours)*	0.01 mg/L
01034	Chromium (total)	0.05 mg/L
01042	Copper	0.005 mg/L
00718	Cyanide (grab) (weak acid dissociable)*	5.0 ug/L
00720	Cyanide (grab) (total)	5.0 ug/L
00951	Fluoride*	0.1 mg/L
01045	Iron (total)	0.5 mg/L
01046	Iron (Dissolved)*	0.5 mg/L
01051	Lead	0.05 mg/L
01055	Manganese	0.5 mg/L
71900	Mercury (effluent grab using USEPA Method 1631 or equivalent)***	1.0 ng/L**
01067	Nickel	0.005 mg/L
00556	Oil (hexane soluble or equivalent) (Grab Sample only)*	5.0 mg/L
32730	Phenols (grab)	0.005 mg/L
01147	Selenium	0.005 mg/L
01077	Silver (total)	0.003 mg/L
01059	Thallium	0.3 mg/L
01092	Zinc	0.025 mg/L

* Influent and effluent only
**1 ng/L = 1 part per trillion.
*** Other approved methods may be used for influent (composite) and sludge

Unless otherwise indicated, concentrations refer to the total amount of the constituent present in all phases, whether solid, suspended or dissolved, elemental or combined including all oxidation states. Where constituents are commonly measured as other than total, the phase is so indicated.

2. The Permittee shall conduct an analysis for the one hundred and ten (110) organic priority pollutants identified in 40 CFR 122 Appendix D, Table II as amended. This monitoring shall be done once per year and reported on monitoring report forms provided by the IEPA and shall consist of the following:

   a. The influent and effluent shall be sampled and analyzed for the one hundred and ten (110) organic priority pollutants. The sampling shall be done during a day when industrial discharges are expected to be occurring at normal to maximum levels.

Special Conditions

Samples for the analysis of acid and base/neutral extractable compounds shall be 24-hour composites.

Five (5) grab samples shall be collected each monitoring day to be analyzed for volatile organic compounds. A single analysis for volatile pollutants (Method 624) may be run for each monitoring day by compositing equal volumes of each grab sample directly in the GC purge and trap apparatus in the laboratory, with no less than one (1) mL of each grab included in the composite.

Wastewater samples must be handled, prepared, and analyzed by GC/MS in accordance with USEPA Methods 624 and 625 of 40 CFR 136 as amended.

b. The sludge shall be sampled and analyzed for the one hundred and ten (110) organic priority pollutants. A sludge sample shall be collected concurrent with a wastewater sample and taken as final sludge.

Sampling and analysis shall conform to USEPA Methods 624 and 625 unless an alternate method has been approved by IEPA.

c. Sample collection, preservation and storage shall conform to approved USEPA procedures and requirements.

3. In addition, the Permittee shall monitor any new toxic substances as defined by the Clean Water Act, as amended, following notification by the IEPA.

4. Permittee shall report any noncompliance with effluent or water quality standards in accordance with Standard Condition 12(e) of this Permit.

5. Analytical detection limits shall be in accordance with 40 CFR 136. Minimum detection limits for sludge analyses shall be in accordance with 40 CFR 503.

SPECIAL CONDITION 9. The Permittee has undergone a Monitoring Reduction review and the influent and effluent sample frequency has been reduced for $CBOD_5$, BOD_5, suspended solids, dissolved oxygen, pH, fecal coliform, chlorine residual and ammonia nitrogen due to sustained compliance. The IEPA will require that the influent and effluent sampling frequency for these parameters be increased to 5 days/week if effluent deterioration occurs due to increased wasteload, operational, maintenance or other problems. The increased monitoring will be required Without Public Notice when a permit modification is received by the Permittee from the IEPA.

SPECIAL CONDITION 10. During January of each year the Permittee shall submit annual fiscal data regarding sewerage system operations to the Illinois Environmental Protection Agency/Division of Water Pollution Control/Compliance Assurance Section. The Permittee may use any fiscal year period provided the period ends within twelve (12) months of the submission date.

Submission shall be on forms provided by IEPA titled "Fiscal Report Form For NPDES Permittees".

SPECIAL CONDITION 11. The Permittee shall conduct biomonitoring of the effluent from Discharge Number(s) 001.

Biomonitoring

1. Acute Toxicity - Standard definitive acute toxicity tests shall be run on at least two trophic levels of aquatic species (fish, invertebrate) representative of the aquatic community of the receiving stream. Testing must be consistent with Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fifth Ed.) EPA/821-R-02-012. Unless substitute tests are pre-approved; the following tests are required:

   a. Fish - 96 hour static LC_{50} Bioassay using fathead minnows (Pimephales promelas).

   b. Invertebrate 48-hour static LC_{50} Bioassay using Ceriodaphnia.

2. Testing Frequency - The above tests shall be conducted using 24-hour composite samples unless otherwise authorized by the IEPA. Samples must be collected in the 18th, 15th, 12th, and 9th month prior to the expiration date of this Permit.

Special Conditions

3. Reporting - Results shall be reported according to EPA/821-R-02-012, Section 12, Report Preparation, and shall be submitted to IEPA, Bureau of Water, Compliance Assurance Section within one week of receipt from the laboratory. Reports are due to the IEPA no later than the 16th, 13th, 10th, and 7th month prior to the expiration date of this Permit.

4. Toxicity Reduction Evaluation - Should the results of the biomonitoring program identify toxicity, the IEPA may require that the Permittee prepare a plan for toxicity reduction evaluation and identification. This plan shall be developed in accordance with Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants, EPA/833B-99/002, and shall include an evaluation to determine which chemicals have a potential for being discharged in the plant wastewater, a monitoring program to determine their presence or absence and to identify other compounds which are not being removed by treatment, and other measures as appropriate. The Permittee shall submit to the IEPA its plan for toxicity reduction evaluation within ninety (90) days following notification by the IEPA. The Permittee shall implement the plan within ninety (90) days or other such date as contained in a notification letter received from the IEPA.

The IEPA may modify this Permit during its term to incorporate additional requirements or limitations based on the results of the biomonitoring. In addition, after review of the monitoring results, the IEPA may modify this Permit to include numerical limitations for specific toxic pollutants. Modifications under this condition shall follow public notice and opportunity for hearing.

SPECIAL CONDITION 12. Discharge Number 002 is an emergency high level bypass. Discharges from this overflow are subject to the following conditions:

(1) Definitions

(I) "Bypass" means the intentional diversion of waste streams from any portion of a treatment facility.

(Ii) "Severe property damage" means substantial physical damage to property, damage to the treatment facilities which causes them to become inoperable, or substantial and permanent loss of natural resources which can reasonably be expected to occur in the absence of a bypass. Severe property damage does not mean economic loss caused by delays in production.

(2) Bypass not exceeding limitations. The Permittee may allow any bypass to occur which does not cause effluent limitations to be exceeded, but only if it also is for essential maintenance to assure efficient operation. These bypasses are not subject to the provisions of paragraphs (3) and (4) of this section.

(3) Notice

(I) Anticipated bypass. If the Permittee knows in advance of the need for a bypass, it shall submit prior notice, if possible at least ten days before the date of the bypass.

(Ii) Unanticipated bypass. The Permittee shall submit notice of an unanticipated bypass as required in Standard Condition 12(e) of this Permit (24-hour notice).

(4) Prohibition of bypass. Bypass is prohibited, and the IEPA may take enforcement action against a Permittee for bypass, unless:

(I) Bypass was unavoidable to prevent loss of life, personal injury, or severe property damage;

(Ii) There was no feasible alternatives to the bypass, such as the use of auxiliary treatment facilities, retention of untreated wastes, or maintenance during normal periods of equipment downtime. This condition is not satisfied if adequate back-up equipment should have been installed in the exercise of reasonable engineering judgment to prevent a bypass which occurred during normal periods of equipment downtime or preventive maintenance; and

(Iii) The Permittee submitted notices as required under Standard Condition 12(e) of this Permit.

(5) Emergency Bypass when discharging, shall be monitored daily by grab sample for BOD_5 and Suspended Solids. The Permittee shall submit the monitoring results on Discharge Monitoring Report forms using one such form for each month in which bypassing occurs. The Permittee shall specify the number of discharges per month that occur and shall report this number in the quantity daily maximum column. The Permittee shall report the highest concentration value of BOD_5 and Suspended Solids discharged in the concentration daily maximum column.

Special Conditions

SPECIAL CONDITION 13. For the duration of this Permit, the Permittee shall determine the quantity of sludge produced by the treatment facility in dry tons or gallons with average percent total solids analysis. The Permittee shall maintain adequate records of the quantities of sludge produced and have said records available for IEPA inspection. The Permittee shall submit to the IEPA, at a minimum, a semi-annual summary report of the quantities of sludge generated and disposed of, in units of dry tons or gallons (average total percent solids) by different disposal methods including but not limited to application on farmland, application on reclamation land, landfilling, public distribution, dedicated land disposal, sod farms, storage lagoons or any other specified disposal method. Said reports shall be submitted to the IEPA by January 31 and July 31 of each year reporting the preceding January thru June and July thru December interval of sludge disposal operations.

Duty to Mitigate. The Permittee shall take all reasonable steps to minimize any sludge use or disposal in violation of this Permit.

Sludge monitoring must be conducted according to test procedures approved under 40 CFR 136 unless otherwise specified in 40 CFR 503, unless other test procedures have been specified in this Permit.

Planned Changes. The Permittee shall give notice to the IEPA on the semi-annual report of any changes in sludge use and disposal.

The Permittee shall retain records of all sludge monitoring, and reports required by the Sludge Permit as referenced in Standard Condition 23 for a period of at least five (5) years from the date of this Permit.

If the Permittee monitors any pollutant more frequently than required by the Sludge Permit, the results of this monitoring shall be included in the reporting of data submitted to the IEPA.

Monitoring reports for sludge shall be reported on the form titled "Sludge Management Reports" to the following address:

Illinois Environmental Protection Agency
Bureau of Water
Compliance Assurance Section
Mail Code #19
1021 North Grand Avenue East
Post Office Box 19276
Springfield, Illinois 62794-9276

SPECIAL CONDITION 14.

AUTHORIZATION OF
COMBINED SEWER AND TREATMENT PLANT DISCHARGES

The IEPA has determined that at least a portion of the collection system consists of combined sewers. References to the collection system and the sewer system refer only to those parts of the system which are owned and operated by the Permittee unless otherwise indicated. The Permittee is authorized to discharge from the overflow(s)/bypass(es) listed below provided the diversion structure is located on a combined sewer and the following terms and conditions are met:

Discharge Number	Location	Receiving Water
A03	Oakland Avenue CSO Treatment Bypass	Sangamon River
A04	South Edward Street CSO Treatment Bypass	Sangamon River
A06	Fairview Park CSO	Stevens Creek
A07	McKinley Avenue CSO Treatment Bypass	Unnamed tributary of Spring Creek
A08	Seventh Ward CSO Treatment Bypass	Sangamon River

Treatment Requirements

1. All combined sewer overflows and treatment plant bypasses shall be given sufficient treatment to prevent pollution and the violation of applicable water quality standards. Sufficient treatment shall consist of the following:

   a. Treatment as described in PCB AS 91-7 and dated June 23, 1992 shall be provided. The terms and conditions of this Board Order are hereby incorporated by reference as if fully set forth herein; and,

Special Conditions

b. Any additional treatment, necessary to comply with applicable water quality standards and the federal Clean Water Act, including any amendments made by the Wet Weather Water Quality Act of 2000.

2. All CSO discharges authorized by this Permit shall be treated, in whole or in part, to the extent necessary to prevent accumulations of sludge deposits, floating debris and solids in accordance with 35 Ill. Adm. Code 302.203 and to prevent depression of oxygen levels below the applicable water quality standards.

3. Overflows during dry weather are prohibited. Dry weather overflows shall be reported to the IEPA pursuant to Standard Condition 12(e) of this Permit (24 hour notice).

4. The collection system shall be operated to optimize transport of wastewater flows and to minimize CSO discharges.

5. The treatment system shall be operated to maximize treatment of wastewater flows.

Nine Minimum Controls

6. The Permittee shall comply with the nine minimum controls contained in the National CSO Control Policy published in the Federal Register on April 19, 1994. The nine minimum controls are:

a. Proper operation and maintenance programs for the sewer system and the CSOs (Compliance with this Item shall be met through the requirements imposed by Paragraph 8 of this Special Condition);

b. Maximum use of the collection system for storage (Compliance with this Item shall be met through the requirements imposed by Paragraphs 1, 4, and 8 of this Special Condition);

c. Review and modification of pretreatment requirements to assure CSO impacts are minimized (Compliance with this Item shall be met through the requirements imposed by Paragraph 9 of this Special Condition);

d. Maximization of flow to the POTW for treatment (Compliance with this Item shall be met through the requirements imposed by Paragraphs 4, 5, and 8 of this Special Condition);

e. Prohibition of CSOs during dry weather (Compliance with this Item shall be met through the requirements imposed by Paragraph 3 of this Special Condition);

f. Control of solids and floatable materials in CSOs (Compliance with this Item shall be met through the requirements imposed by Paragraphs 2 and 8 of this Special Condition);

g. Pollution prevention programs which focus on source control activities (Compliance with this Item shall be met through the requirements imposed by Paragraph 6 of this Special Condition, **See Below**);

h. Public notification to ensure that citizens receive adequate information regarding CSO occurrences and CSO impacts (Compliance with this Item shall be met through the requirements imposed by Paragraph 12 of this Special Condition); and,

i. Monitoring to characterize impacts and efficiency of CSO controls (Compliance with this Item shall be met through the requirements imposed by Paragraphs 10 and 11 of this Special Condition).

Special Conditions

A pollution prevention plan (PPP) shall be developed by the Permittee unless one has already been prepared for this collection system. Any previously-prepared PPP shall be reviewed, and revised if necessary, by the Permittee to address the items contained in Chapter 8 of the U.S. EPA guidance document, Combined Sewer Overflows, Guidance For Nine Minimum Controls, and any items contained in previously-sent review documents from the IEPA concerning the PPP. Combined Sewer Overflows, Guidance For Nine Minimum Controls is available on line at http://www.epa.gov/NPDES/pubs/owm0030.pdf. The PPP (or revised PPP) shall be presented to the general public at a public information meeting conducted by the Permittee within nine (9) months of the effective date of this Permit. The Permittee shall submit documentation that the pollution prevention plan complies with the requirements of this Permit and that the public information meeting was held. Such documentation shall be submitted to the IEPA within twelve (12) months of the effective date of this Permit and shall include a summary of all significant issues raised by the public, the Permittee's response to each issue, and two (2) copies of the "CSO Pollution Prevention Plan Certification" one (1) with original signatures. This certification form is available online at http://www.epa.state.il.us/water/permits/waste-water/forms/cso-pol-prev.pdf. Following the public meeting, the Permittee shall implement the pollution prevention plan within one (1) year and shall maintain a current pollution prevention plan, updated to reflect system modifications, on file at the sewage treatment works or other acceptable location and made available to the public. The pollution prevention plan shall be submitted to the IEPA upon written request.

Sensitive Area Considerations

7. Pursuant to Section II.C.3 of the federal CSO Control Policy of 1994, sensitive areas are any water likely to be impacted by a CSO discharge which meet one or more of the following criteria: (1) designated as an Outstanding National Resource Water; (2) found to contain shellfish beds; (3) found to contain threatened or endangered aquatic species or their habitat; (4) used for primary contact recreation; or, (5) within the protection area for a drinking water intake structure.

   The IEPA has tentatively determined that none of the outfalls listed in this Special Condition discharge to sensitive areas. However, if information becomes available that causes the IEPA to reverse this determination, the IEPA will notify the Permittee in writing. Within three (3) months of the date of notification, or such other date contained in the notification letter, the Permittee shall submit two (2) copies of either a schedule to relocate, control, or treat discharges from these outfalls. If none of these options are possible, the Permittee shall submit adequate justification at that time as to why these options are not possible. Such justification shall be in accordance with Section II.C.3 of the National CSO Control Policy.

Operational and Maintenance Plans

8. The IEPA reviewed and accepted a CSO operational and maintenance plan "CSO O&M plan" on February 1, 2000 prepared for this sewerage system. The Permittee shall review and revise, if needed, the CSO O&M plan to reflect system changes.

   The CSO O&M plan shall be presented to the general public at a public information meeting conducted by the Permittee within nine (9) months of the effective date of this Permit. The Permittee shall submit documentation that the CSO O&M plan complies with the requirements of this Permit and that the public information meeting was held. Such documentation shall be submitted to the IEPA within twelve (12) months of the effective date of this Permit and shall include a summary of all significant issues raised by the public, the Permittee's response to each issue, and two (2) copies of the "CSO Operational Plan Checklist and Certification", one (1) with original signatures. Copies of the "CSO Operational Plan Checklist and Certification" are available online at http://www.epa.state.il.us/water/permits/waste-water/forms/cso-checklist.pdf. Following the public meeting, the Permittee shall implement the CSO O&M plan within one (1) year and shall maintain a current CSO O&M plan, updated to reflect system modifications, on file at the sewage treatment works or other acceptable location and made available to the public. The CSO O&M plan shall be submitted to the IEPA upon written request.
   The objectives of the CSO O&M plan are to reduce the total loading of pollutants and floatables entering the receiving stream and to ensure that the Permittee ultimately achieves compliance with water quality standards. These plans, tailored to the local governments's collection and waste treatment systems, shall include mechanisms and specific procedures where applicable to ensure:

   a. Collection system inspection on a scheduled basis;

   b. Sewer, catch basin, and regulator cleaning and maintenance on a scheduled basis;

   c. Inspections are made and preventive maintenance is performed on all pump/lift stations;

   d. Collection system replacement, where necessary;

   e. Detection and elimination of illegal connections;

f. Detection, prevention, and elimination of dry weather overflows;

g. The collection system is operated to maximize storage capacity and the combined sewer portions of the collection system are operated to delay storm entry into the system; and,

h. The treatment and collection systems are operated to maximize treatment.

Sewer Use Ordinances

9. The Permittee, within six (6) months of the effective date of this Permit, shall review and where necessary, modify its existing sewer use ordinance to ensure it contains provisions addressing the conditions below. If no ordinance exists, such ordinance shall be developed and implemented within six (6) months from the effective date of this Permit. Upon completion of the review of the sewer use ordinance(s), the Permittee shall submit two (2) copies of a completed "Certification of Sewer Use Ordinance Review", one (1) with original signatures. Copies of the certification form can be obtained on line at http://www.epa.state.il.us/water/permits/waste-water/forms/sewer-use.pdf. The Permittee shall submit copies of the sewer use ordinance(s) to the IEPA upon written request. Sewer use ordinances are to contain specific provisions to:

a. prohibit introduction of new inflow sources to the sanitary sewer system;

b. require that new construction tributary to the combined sewer system be designed to minimize and/or delay inflow contribution to the combined sewer system;

c. require that inflow sources on the combined sewer system be connected to a storm sewer, within a reasonable period of time, if a storm sewer becomes available;

d. provide that any new building domestic waste connection shall be distinct from the building inflow connection, to facilitate disconnection if a storm sewer becomes available;

e. assure that CSO impacts from non-domestic sources are minimized by determining which non-domestic discharges, if any, are tributary to CSOs and reviewing, and, if necessary, modifying the sewer use ordinance to control pollutants in these discharges; and,

f. notify the owners of all publicly owned systems with combined sewers tributary to the Permittee's collection system of their obligations to have procedures in place adequate to ensure that the objectives, mechanisms, and specific procedures given in Paragraph 8 of this Special Condition are achieved.

The Permittee shall enforce the applicable sewer use ordinances.

Long-Term Control Planning and Compliance with Water Quality Standards

10. a. Pursuant to Section 301 of the federal Clean Water Act, 33 U.S.C. § 1311 and 40 CFR § 122.4, discharges from the CSOs, including the outfalls listed in this Special Condition and any other outfall listed as a "Treated Combined Sewage Outfall", shall not cause or contribute to violations of applicable water quality standards or cause use impairment in the receiving waters. In addition, discharges from CSOs shall comply with all applicable parts of 35 Ill. Adm. Code 306.305(a), (b), (c), and (d).

Special Conditions

b. Based on available information, it appears that the CSOs authorized in this Permit meet the criteria of Section II.C.4.a.i of the federal CSO Control Policy of 1994 (Policy), not more than four overflow events per year, and are presumed to meet the water quality-based requirements of the federal Clean Water Act. Pursuant to Section I.C.1 and Section II.C.9 of the Policy, the Permittee shall develop a post-construction water quality monitoring program adequate to verify compliance with water quality standards and to verify protection of designated uses in the receiving water(s) and to ascertain the effectness of CSO controls. This program shall contain a plan that details the monitoring protocols to be followed, including any necessary effluent and ambient monitoring, and if appropriate, other monitoring protocols such as biological assessments, whole effluent toxicity testing, and sediment sampling. This plan shall be presented to the public at an informational meeting within nine (9) months of the effective date of this Permit. Within twelve (12) months of the effective date of this Permit, the Permittee shall submit a summary of all significant issues raised by the public, the Permittee's response to each issue, and two (2) copies of the final plan (revised following the public meeting, if necessary) implementing the post-construction monitoring program. The post-construction monitoring plan shall be implemented within six (6) months of the date of IEPA approval. The Permittee shall respond to an IEPA review letter in writing within ninety (90) days of the date of such an initial review letter and within thirty (30) days of any subsequent review letter(s), if any. Within thirty (30) months of the approval of the plan, the results shall be submitted to the IEPA along with recommendations and conclusions as to whether or not the discharges from any of the CSOs (treated or untreated) authorized by this Permit are causing or contributing to violations of applicable water quality standards or causing use impairment in the receiving water(s).

c. Should the results of the post-construction water quality monitoring plan or if information becomes available that causes IEPA to conclude that the discharges from any of the CSOs (treated or untreated) authorized to discharge under this Permit are causing or contributing to violations of water quality standards or are causing use impairment in the receiving water(s), the IEPA will notify the Permittee in writing. Upon receiving such notification, the Permittee shall develop and implement a CSO Long-Term Control Plan (LTCP) for assuring that the discharges from the CSOs (treated or untreated) authorized in this Permit comply with the provisions of Paragraph 10.a above. The LTCP shall contain all applicable elements of Paragraph 10.d below including a schedule for implementation and provisions for re-evaluating compliance with applicable standards and regulations after complete implementation. Two (2) copies of the LTCP shall be submitted to the IEPA within twelve (12) months of receiving the IEPA written notice. The LTCP shall be:

1. Consistent with Section II.C.4.a.i of the Policy; or,
2. Consistent with either Section II.C.4.a.ii, Section II.C.4.a.iii, or Section II.C.4.b of the Policy and be accompanied by data sufficient to demonstrate that the LTCP, when completely implemented, will be sufficient to meet water quality standards.

d. Pursuant to the Policy, the required components of the LTCP include the following:

1. Characterization, monitoring, and modeling of the Combined Sewer System (CSS);
2. Consideration of Sensitive Areas;
3. Evaluation of alternatives;
4. Cost/Performance considerations;
5. Revised CSO Operational Plan;
6. Maximizing treatment at the treatment plant;
7. Implementation schedule;
8. Post-Construction compliance monitoring program; and
9. Public participation.

Following submittal of the LTCP, the Permittee shall respond to any initial IEPA review letter in writing within ninety (90) days of the date of such a review letter, and within thirty (30) days of any subsequent review letter(s), if any. Implementation of the LTCP shall be as indicated by IEPA in writing or other enforceable mechanism.

Monitoring, Reporting and Notification Requirements

11. The Permittee shall monitor the frequency of discharge (number of discharges per month) and estimate the duration (in hours) of each discharge from each outfall listed in this Special Condition. Estimates of storm duration and total rainfall shall be provided for each storm event.

Special Conditions

For frequency reporting, all discharges from the same storm, or occurring within 24 hours, shall be reported as one. The date that a discharge commences shall be recorded for each outfall. Reports shall be in the form specified by the IEPA and on forms provided by the IEPA. These forms shall be submitted to the IEPA monthly with the DMRs and covering the same reporting period as the DMRs. Parameters (other than flow frequency), if required in this Permit, shall be sampled and reported as indicated in the transmittal letter for such report forms.

12. A public notification program in accordance with Section II.B.8 of the federal CSO Control Policy of 1994 shall be developed employing a process that actively informs the affected public. The program shall include at a minimum public notification of CSO occurrences and CSO impacts, with consideration given to including mass media and/or Internet notification. The Permittee shall also consider posting signs in waters likely to be impacted by CSO discharges at the point of discharge and at points where these waters are used for primary contact recreation. Provisions shall be made to include modifications of the program when necessary and notification to any additional member of the affected public. The program shall be presented to the general public at a public information meeting conducted by the Permittee. The Permittee shall conduct the public information meeting within nine (9) months of the effective date of this Permit. The Permittee shall submit documentation that the public information meeting was held, shall submit a summary of all significant issues raised by the public and the Permittee's response to each issue and shall identify any modifications to the program as a result of the public information meeting. The Permittee shall submit the public information meeting documentation to the IEPA and implement the public notification program within twelve (12) months of the effective date of this Permit. The Permittee shall submit copies of the public notification program to the IEPA upon written request.

13. If any of the CSO discharge points listed in this Special Condition are eliminated, or if additional CSO discharge points, not listed in this Special Condition, are discovered, the Permittee shall notify the IEPA in writing within one (1) month of the respective outfall elimination or discovery. Such notification shall be in the form of a request for the appropriate modification of this NPDES Permit.

Summary of Compliance Dates in this CSO Special Condition

14. The following summarizes the dates that submittals contained in this Special Condition are due at the IEPA (unless otherwise indicated):

Submission of CSO Monitoring Data (Paragraph 11)	15th of every month
Elimination of a CSO or Discovery of Additional CSO Locations (Paragraph 13)	1 month from discovery or elimination
Control (or Justification for No Control) of CSOs to Sensitive Areas (Paragraph 7)	3 months from IEPA notification
Certification of Sewer Use Ordinance Review (Paragraph 9)	6 months from the effective date of this Permit
Implement Post-Construction Monitoring Plan (Paragraph 10) **No Submittal Due with this Milestone**	6 months from the date of IEPA plan approval
Conduct Pollution Prevention, OMP, Post-Construction Monitoring Plan and PN Public Information Meeting (Paragraphs, 6, 8, 10 and 12) **No Submittal Due with this Milestone**	9 months from the effective date of this Permit
Submit Pollution Prevention Certification, OMP Certification, Post-Construction Monitoring Plan and PN Information Meeting Summary (Paragraphs, 6, 8, 10 and 12)	12 months from the effective date of this Permit
Submit CSO Long-Term Control Plan (Paragraph 10)	12 months from the date of IEPA notification
Submit Results of Post-Construction Monitoring Plan (Paragraph 10)	30 months from the date of IEPA plan approval

Special Conditions

All submittals listed in this Special Condition can be mailed to the following address:

Illinois Environmental Protection Agency
Division of Water Pollution Control
1021 North Grand Avenue East
Post Office Box 19276
Springfield, Illinois 62794-9276

Attention: CSO Coordinator, Compliance Assurance Section

All submittals hand carried shall be delivered to 1021 North Grand Avenue East.

Reopening and Modifying this Permit

15. The IEPA may initiate a modification for this Permit at any time to include requirements and compliance dates which have been submitted in writing by the Permittee and approved by the IEPA, or other requirements and dates which are necessary to carry out the provisions of the Illinois Environmental Protection Act, the Clean Water Act, or regulations promulgated under those Acts. Public Notice of such modifications and opportunity for public hearing shall be provided.

SPECIAL CONDITION 15. The Permittee shall record monitoring results on Discharge Monitoring Report (DMR) Forms using one such form for each outfall each month.

In the event that an outfall does not discharge during a monthly reporting period, the DMR Form shall be submitted with no discharge indicated.

The Permittee may choose to submit electronic DMRs (eDMRs) instead of mailing paper DMRs to the IEPA. More information, including registration information for the eDMR program, can be obtained on the IEPA website, http://www.epa.state.il.us/water/edmr/index.html.

The completed Discharge Monitoring Report forms shall be submitted to IEPA no later than the 15th day of the following month, unless otherwise specified by the permitting authority.

Permittees not using eDMRs shall mail Discharge Monitoring Reports with an original signature to the IEPA at the following address:

Illinois Environmental Protection Agency
Division of Water Pollution Control
1021 North Grand Avenue East
Post Office Box 19276
Springfield, Illinois 62794-9276

Attention: Compliance Assurance Section, Mail Code # 19

SPECIAL CONDITION 16. The Permittee has collected data in support of developing a site-specific metals translator for nickel and zinc. The IEPA has reviewed the sample data and has revised effluent limitations for these parameters based on the metal translator determined from the collected data.

NPDES Permit No. IL0028321

Special Conditions

SPECIAL CONDITION 17.

Project Description: Compliance with Nickel and Zinc Water Quality Standards

Thirty-six (36) months from the effective date of this Permit the following nickel and zinc limits and monitoring requirements found on page two of this permit shall become effective:

	Load Limits lbs/day DAF (DMF)*		Concentration Limits mg/L	
	Monthly Avg.	Daily Max.	Monthly Avg.	Daily Max.
Zinc	26 (78)	142 (434)	0.075	0.416
Nickel	5.1 (16)		0.015	

*Load limits based on design maximum flow shall apply only when flow exceeds the design average flow.

The Permittee shall complete the project described above in accordance with the following schedule:

(1)	Interim Report on effluent and stream sampling to date and what measures are necessary to comply with Final Nickel and Zinc Limitations	6 months from the effective date of this Permit
(2)	Interim Report	12 months from the effective date of this Permit
(3)	Interim Report	18 months from the effective date of this Permit
(4)	Interim Report	24 months from the effective date of this Permit
(5)	Interim Report	30 months from the effective date of this Permit
(6)	Permittee Achieves Compliance with Final Nickel and Zinc Effluent Limitations	36 months from the effective date of this Permit

This Permit may be modified, with Public Notice, to include revised compliance dates set out in this Permit that are superseded or supplemented by compliance dates in judicial orders, Pollution Control Board orders or grant agreements. Prior to such permit modification, the revised dates in the appropriate orders or grant agreements shall govern the Permittee's compliance.

In addition, the IEPA may initiate a modification of the construction schedule set forth in this Permit at any time, to include other dates which are necessary to carry out the provisions of the Illinois Environmental Protection Act, the Federal Clean Water Act or regulations promulgated under those Acts or compliance dates which have been submitted in writing by the Permittee and approved by the IEPA. Public Notice of such modifications and opportunity for public hearing shall be provided consistent with 40 CFR § 122.63.

REPORTING

The Permittee shall submit a report no later than fourteen (14) days following the completion dates indicated for each numbered item in the compliance schedule, indicating, a) the date the item was completed, or b) that the item was not completed. All reports shall be submitted to IEPA at the following address:

Illinois Environmental Protection Agency
Division of Water Pollution Control
1021 North Grand Avenue East
Post Office box 19276
Springfield, Illinois 62794-9276

Attention: Compliance Assurance Section, Mail Code # 19

NPDES Permit No. IL0028321

Special Conditions

SPECIAL CONDITION 18.

STORM WATER POLLUTION PREVENTION PLAN (SWPPP)

1. A storm water pollution prevention plan shall be developed by the permittee and submitted to the Agency for each facility covered by this permit. The plan shall identify potential sources of pollution which may be expected to affect the quality of storm water discharges associated with the industrial activity at the facility. In addition, the plan shall describe and ensure the implementation of practices which are to be used to reduce the pollutants in storm water discharges associated with industrial activity at the facility and to assure compliance with the terms and conditions of this permit. An electronic copy of the plan shall be submitted to the Agency at the following email address: epa.indilr00swppp@illinois.gov. The permittee shall submit any modified plan to the Agency, when such modification includes substantive changes to the plan or modification is made to the plan for compliance with this permit.

   a. Waters not classified as Impaired pursuant to Section 303(d) of the Clean Water Act

      Unless otherwise specified by federal regulation, the storm water pollution prevention plan shall be designed for a storm event equal to or greater than a 25-year 24-hour rainfall event.

   b. Waters classified as Impaired pursuant to Section 303(d) of the Clean Water Act

      For any site which discharges directly to an impaired water identified in the Agency's 303(d) listing, and if any parameter in the subject discharge has been identified as the cause of impairment, the storm water pollution prevention plan shall be designed for a storm event equal to or greater than a 25-year 24-hour rainfall event. If required by federal regulations, the storm water pollution prevention plan shall adhere to a more restrictive design criteria.

2 Plans for new facilities shall be completed prior to submitting an NOI to be covered under this permit. An electronic copy of the storm water pollution prevention plan shall be submitted to the Agency at the following email address: epa.indilr00swppp@illinois.gov. Plans shall provide for compliance with the terms of this permit prior to operation of any industrial activity to be covered under this permit. [Note: If the plan has already been required to be developed under a previous permit it shall be maintained in accordance with all requirements of this special condition.]. The owner or operator of an existing facility with storm water discharges covered by this permit shall make a copy of the plan available to the Agency at any reasonable time upon request.

   Facilities which discharge to a municipal separate storm sewer system shall also make a copy available to the operator of the municipal system at any reasonable time upon request.

3. The permittee may be notified by the Agency at any time that the plan does not meet the requirements of this permit. After such notification, the permittee shall make changes to the plan and shall submit a revised plan to the Agency, with the requested changes that have been made. Unless otherwise provided, the permittee shall have 30 days after such notification to make the changes.

4. The discharger shall amend the plan whenever there is a change in construction, operation, or maintenance which may affect the discharge of significant quantities of pollutants to the waters of the State or if a facility inspection required by paragraph E.8.of this permit indicates that an amendment is needed. The plan should also be amended if the discharger is in violation of any conditions of this permit, or has not achieved the general objectives of controlling pollutants in storm water discharges. Amendments to the plan shall be made within 30 days of any proposed construction or operational changes at the facility, and shall be submitted to the Agency.

5. The plan shall provide a description of potential sources which may be expected to add significant quantities of pollutants to storm water discharges, or which may result in non-storm water discharges from the facility. The plan shall include, at a minimum, the following items:

   a. A topographic map extending one-quarter mile beyond the property boundaries of the facility, showing: the facility, surface water bodies, wells (including injection wells), seepage pits, infiltration ponds, and the discharge points where the facility's storm water discharges to a municipal storm drain system or other water body. The requirements of this paragraph may be included on the site map if appropriate. Any map or portion of map may be withheld for security reasons.

b. A site map showing:

i. The storm water conveyance and discharge structures;

ii. An outline of the storm water drainage areas for each storm water discharge point;

iii. Paved areas and buildings;

iv. Areas used for outdoor manufacturing, storage, or disposal of significant materials, including activities that generate significant quantities of dust or particulates;

v. Location of existing or future storm water structural control measures/practices (dikes, coverings, detention facilities, etc.);

vi. Surface water locations and/or municipal storm drain locations;

vii. Areas of existing and potential soil erosion;

viii. Vehicle service areas;

ix. Material loading, unloading, and access areas;

x. Areas under Items iv and ix above may be withheld from the site map for security reasons.

c. A narrative description of the following:

i. The nature of the industrial activities conducted at the site, including a description of significant materials that are treated, stored or disposed of in a manner to allow exposure to storm water;

ii. Materials, equipment, and vehicle management practices employed to minimize contact of significant materials with storm water discharges;

iii. Existing or future structural and non-structural control measures/practices to reduce pollutants in storm water discharges;

iv. Industrial storm water discharge treatment facilities;

v. Methods of onsite storage and disposal of significant materials.

d. A list of the types of pollutants that have a reasonable potential to be present in storm water discharges in significant quantities. Also provide a list of any pollutant that is listed as impaired in the most recent 303(d) report.

e. An estimate of the size of the facility in acres or square feet, and the percent of the facility that has impervious areas such as pavement or buildings.

f. A summary of existing sampling data describing pollutants in storm water discharges.

6. The plan shall describe the storm water management controls which will be implemented by the facility. The appropriate controls shall reflect identified existing and potential sources of pollutants at the facility. The description of the storm water management controls shall include:

a. Storm Water Pollution Prevention Personnel - Identification by job titles, direct telephone numbers and email addresses of the individuals who are responsible for developing, implementing, and revising the plan.

b. Preventive Maintenance - Procedures and frequencies for inspection and maintenance of storm water conveyance system devices such as oil/water separators, catch basins, etc., and inspection and testing of plant equipment and systems that could fail and result in discharges of pollutants to storm water.

Special Conditions

c. Good Housekeeping - Good housekeeping requires the maintenance of clean, orderly facility areas that discharge storm water. Material handling areas shall be inspected and cleaned to reduce the potential for pollutants to enter the storm water conveyance system.

d. Spill Prevention and Response - Identification of areas where significant materials can spill into or otherwise enter the storm water conveyance systems and their accompanying drainage points. Specific material handling procedures, storage requirements, spill cleanup equipment and procedures should be identified, as appropriate. Internal notification procedures for spills of significant materials should be established.

e. Storm Water Management Practices - Storm water management practices are practices other than those which control the source of pollutants. They include measures such as installing oil and grit separators, diverting storm water into retention basins, etc. Based on assessment of the potential of various sources to contribute pollutants, measures to remove pollutants from storm water discharge shall be implemented. In developing the plan, the following management practices shall be considered:

i. Containment - Storage within berms or other secondary containment devices to prevent leaks and spills from entering storm water runoff. To the maximum extent practicable, storm water discharged from any area where material handling equipment
or activities, raw materials, intermediate products, final products, waste materials, by-products, or industrial machinery are exposed to storm water should not enter vegetated areas or surface waters or infiltrate into the soil unless adequate treatment is provided.

ii. Oil & Grease Separation - Oil/water separators, booms, skimmers or other methods to minimize oil contaminated storm water discharges.

iii. Debris & Sediment Control - Screens, booms, sediment ponds or other methods to reduce debris and sediment in storm water discharges.

iv. Waste Chemical Disposal - Waste chemicals such as antifreeze, degreasers and used oils shall be recycled or disposed of in an approved manner and in a way which prevents them from entering storm water discharges.

v. Storm Water Diversion - Storm water diversion away from materials manufacturing, storage and other areas of potential storm water contamination. Minimize the quantity of storm water entering areas where material handling equipment or activities, raw materials, intermediate products, final products, waste materials, by-products, or industrial machinery are exposed to storm water using green infrastructure techniques where practicable in the areas outside the exposure area, and otherwise divert storm water away from the exposure area.

vi. Covered Storage or Manufacturing Areas - Covered fueling operations, materials manufacturing and storage areas to prevent contact with storm water.

vii. Mercury Switch Removal and Recycling – Mercury-containing convenience lighting switches and anti-lock brake assemblies shall be removed from vehicles, and recycled in an approved manner, in a way which prevents mercury from entering the storm water discharges.

viii. Storm Water Reduction – Install vegetation on roofs of buildings within and adjacent to the exposure area to detain and evapotranspirate runoff where the precipitation falling on the roof is not exposed to contaminants, to minimize storm water runoff; capture storm water in devices that minimize the amount of storm water runoff and use this water as appropriate based on quality.

f. Sediment and Erosion Prevention - The plan shall identify areas which due to topography, activities, or other factors, have a high potential for significant soil erosion. The plan shall describe measures to limit erosion.

g. Employee Training - Employee training programs shall inform personnel at all levels of responsibility of the components and goals of the storm water pollution prevention plan. Training should address topics such as spill response, good housekeeping and material management practices. The plan shall identify periodic dates for such training.

h. Inspection Procedures - Qualified plant personnel shall be identified to inspect designated equipment and plant areas. A tracking or follow-up procedure shall be used to ensure appropriate response has been taken in response to an inspection. Inspections and maintenance activities shall be documented and recorded.

7. Non-Storm water Discharges - The plan shall include a certification that the discharge has been tested or evaluated for the presence of non-storm water discharges. The certification shall include a description of any tests for the presence of non-storm water discharges, the methods used, the dates of the testing, and any onsite drainage points that were observed during the testing. Any facility that is unable to provide this certification must describe the procedure of any test conducted for the presence of non-storm water discharges, the test results, potential sources of non-storm water discharges to the storm sewer, and why adequate tests for such storm sewers were not feasible. Except as provided in C.1. b., discharges not comprised entirely of storm water are not authorized by this permit.

8. Quarterly Visual Observation of Discharges – The requirements and procedures for quarterly visual observations are applicable to all facilities covered under this permit, regardless of your sector of industrial activity.

   a. You must perform and document a quarterly visual observation of a storm water discharge associated with industrial activity from each outfall. The visual observation must be made during daylight hours. If no storm event resulted in runoff during daylight hours from the facility during a monitoring quarter, you are excused from the visual observation requirement for that quarter, provided you document in your records that no runoff occurred. You must sign and certify the documentation.

   b. Your visual observation must be made on samples collected as soon as practical, but not to exceed 1 hour of when the runoff or snowmelt begins discharging from your facility. All samples must be collected from a storm event discharge that is greater than 0.1 inch in magnitude and that occurs at least 72 hours from the previously measurable (greater than 0.1 inch rainfall) storm event. The observation must document: color, odor, clarity, floating solids, settled solids, suspended solids, foam, oil sheen, and other obvious indicators of storm water pollution. If visual observations indicate any unnatural color, odor, turbidity, floatable material, oil sheen or other indicators of storm water pollution, the permittee shall obtain a sample and monitor for the parameter or the list of pollutants in Part E.5.d.

   c. You must maintain your visual observation reports onsite with the SWPPP. The report must include the observation date and time, inspection personnel, nature of the discharge (i.e., runoff or snow melt), visual quality of the storm water discharge (including observations of color, odor, clarity, floating solids, settled solids, suspended solids, foam, oil sheen, and other obvious indicators of storm water pollution), and probable sources of any observed storm water contamination.

   d. You may exercise a waiver of the visual observation requirement at a facility that is inactive and unstaffed, as long as there are no industrial materials or activities exposed to storm water. If you exercise this waiver, you must maintain a certification with your SWPPP stating that the site is inactive and unstaffed, and that there are no industrial materials or activities exposed to storm water.

   e. Representative Outfalls – If your facility has two or more outfalls that you believe discharge substantially identical effluents, based on similarities of the industrial activities, significant materials, size of drainage areas, and storm water management practices occurring within the drainage areas of the outfalls, you may conduct visual observation of the discharge at just one of the outfalls and report that the results also apply to the substantially identical outfall(s).

   f. The visual observation documentation shall be made available to the Agency and general public upon written request.

9. The permittee shall conduct an annual facility inspection to verify that all elements of the plan, including the site map, potential pollutant sources, and structural and non-structural controls to reduce pollutants in industrial storm water discharges are accurate. Observations that require a response and the appropriate response to the observation shall be retained as part of the plan. Records documenting significant observations made during the site inspection shall be submitted to the Agency in accordance with the reporting requirements of this permit.

10. This plan should briefly describe the appropriate elements of other program requirements, including Spill Prevention Control and Countermeasures (SPCC) plans required under Section 311 of the CWA and the regulations promulgated thereunder, and Best Management Programs under 40 CFR 125.100.

11. The plan is considered a report that shall be available to the public at any reasonable time upon request.

12. The plan shall include the signature and title of the person responsible for preparation of the plan and include the date of initial preparation and each amendment thereto.

13. Facilities which discharge storm water associated with industrial activity to municipal separate storm sewers may also be subject to additional requirements imposed by the operator of the municipal system.

REPORTING

1. The facility shall submit an electronic copy of the annual inspection report to the Illinois Environmental Protection Agency. The report shall include results of the annual facility inspection which is required by Part 9 of the Storm Water Pollution Prevention Plan of this permit. The report shall also include documentation of any event (spill, treatment unit malfunction, etc.) which would require an inspection, results of the inspection, and any subsequent corrective maintenance activity. The report shall be completed and signed by the authorized facility employee(s) who conducted the inspection(s). The annual inspection report is considered a public document that shall be available to the public at any reasonable time upon request.

2. The first report shall contain information gathered during the one year time period beginning with the effective date of coverage under this permit and shall be submitted no later than 60 days after this one year period has expired. Each subsequent report shall contain the previous year's information and shall be submitted no later than one year after the previous year's report was due.

3. If the facility performs inspections more frequently than required by this permit, the results shall be included as additional information in the annual report.

4. The permittee shall retain the annual inspection report on file at least 3 years. This period may be extended by request of the Illinois Environmental Protection Agency at any time.

Annual inspection reports shall be submitted to the following email and office addresses: epa.indannualinsp@illinois.gov

Illinois Environmental Protection Agency
Division of Water Pollution Control
Compliance Assurance Section #19
Annual Inspection Report
P.O. Box 19276
Springfield, Illinois 62794-9276

5. Any permittee shall notify any regulated small municipal separate storm water system owner (MS4 Community) that they have received coverage of a general ILR00 permit. The permittee shall submit any SWPPP or any annual inspection to the MS4 community upon request by the MS4 community.

Attachment H

Standard Conditions

Definitions

Act means the Illinois Environmental Protection Act, 415 ILCS 5 as Amended.

Agency means the Illinois Environmental Protection Agency.

Board means the Illinois Pollution Control Board.

Clean Water Act (formerly referred to as the Federal Water Pollution Control Act) means Pub. L 92-500, as amended. 33 U.S.C. 1251 et seq.

NPDES (National Pollutant Discharge Elimination System) means the national program for issuing, modifying, revoking and reissuing, terminating, monitoring and enforcing permits, and imposing and enforcing pretreatment requirements, under Sections 307, 402, 318 and 405 of the Clean Water Act.

USEPA means the United States Environmental Protection Agency.

Daily Discharge means the discharge of a pollutant measured during a calendar day or any 24-hour period that reasonably represents the calendar day for purposes of sampling. For pollutants with limitations expressed in units of mass, the "daily discharge" is calculated as the total mass of the pollutant discharged over the day. For pollutants with limitations expressed in other units of measurements, the "daily discharge" is calculated as the average measurement of the pollutant over the day.

Maximum Daily Discharge Limitation (daily maximum) means the highest allowable daily discharge.

Average Monthly Discharge Limitation (30 day average) means the highest allowable average of daily discharges over a calendar month, calculated as the sum of all daily discharges measured during a calendar month divided by the number of daily discharges measured during that month.

Average Weekly Discharge Limitation (7 day average) means the highest allowable average of daily discharges over a calendar week, calculated as the sum of all daily discharges measured during a calendar week divided by the number of daily discharges measured during that week.

Best Management Practices (BMPs) means schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the State. BMPs also include treatment requirements, operating procedures, and practices to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage.

Aliquot means a sample of specified volume used to make up a total composite sample.

Grab Sample means an individual sample of at least 100 milliliters collected at a randomly-selected time over a period not exceeding 15 minutes.

24 Hour Composite Sample means a combination of at least 8 sample aliquots of at least 100 milliliters, collected at periodic intervals during the operating hours of a facility over a 24-hour period.

8 Hour Composite Sample means a combination of at least 3 sample aliquots of at least 100 milliliters, collected at periodic intervals during the operating hours of a facility over an 8-hour period.

Flow Proportional Composite Sample means a combination of sample aliquots of at least 100 milliliters collected at periodic intervals such that either the time interval between each aliquot or the volume of each aliquot is proportional to either the stream flow at the time of sampling or the total stream flow since the collection of the previous aliquot.

(1) **Duty to comply.** The permittee must comply with all conditions of this permit. Any permit noncompliance constitutes a violation of the Act and is grounds for enforcement action, permit termination, revocation and reissuance, modification, or for denial of a permit renewal application. The permittee shall comply with effluent standards or prohibitions established under Section 307(a) of the Clean Water Act for toxic pollutants within the time provided in the regulations that establish these standards or prohibitions, even if the permit has not yet been modified to incorporate the requirement.

(2) **Duty to reapply.** If the permittee wishes to continue an activity regulated by this permit after the expiration date of this permit, the permittee must apply for and obtain a new permit. If the permittee submits a proper application as required by the Agency no later than 180 days prior to the expiration date, this permit shall continue in full force and effect until the final Agency decision on the application has been made.

(3) **Need to halt or reduce activity not a defense.** It shall not be a defense for a permittee in an enforcement action that it would have been necessary to halt or reduce the permitted activity in order to maintain compliance with the conditions of this permit.

(4) **Duty to mitigate.** The permittee shall take all reasonable steps to minimize or prevent any discharge in violation of this permit which has a reasonable likelihood of adversely affecting human health or the environment.

(5) **Proper operation and maintenance.** The permittee shall at all times properly operate and maintain all facilities and systems of treatment and control (and related appurtenances) which are installed or used by the permittee to achieve compliance with conditions of this permit. Proper operation and maintenance includes effective performance, adequate funding, adequate operator staffing and training, and adequate laboratory and process controls, including appropriate quality assurance procedures. This provision requires the operation of back-up, or auxiliary facilities, or similar systems only when necessary to achieve compliance with the conditions of the permit.

(6) **Permit actions.** This permit may be modified, revoked and reissued, or terminated for cause by the Agency pursuant to 40 CFR 122.62. The filing of a request by the permittee for a permit modification, revocation and reissuance, or termination, or a notification of planned changes or anticipated noncompliance, does not stay any permit condition.

(7) **Property rights.** This permit does not convey any property rights of any sort, or any exclusive privilege.

(8) **Duty to provide information.** The permittee shall furnish to the Agency within a reasonable time, any information which the Agency may request to determine whether cause exists for modifying, revoking and reissuing, or terminating this permit, or to determine compliance with the permit. The permittee shall also furnish to the Agency, upon request, copies of records required to be kept by this permit.

(9) **Inspection and entry.** The permittee shall allow an authorized representative of the Agency, upon the presentation of credentials and other documents as may be required by law, to:

(a) Enter upon the permittee's premises where a regulated facility or activity is located or conducted, or where records must be kept under the conditions of this permit;

(b) Have access to and copy, at reasonable times, any records that must be kept under the conditions of this permit;

(c) Inspect at reasonable times any facilities, equipment (including monitoring and control equipment), practices, or operations regulated or required under this permit; and

(d) Sample or monitor at reasonable times, for the purpose of assuring permit compliance, or as otherwise authorized by the Act, any substances or parameters at any location.

(10) **Monitoring and records.**

(a) Samples and measurements taken for the purpose of monitoring shall be representative of the monitored activity.

(b) The permittee shall retain records of all monitoring information, including all calibration and maintenance records, and all original strip chart recordings for continuous monitoring instrumentation, copies of all reports required by this permit, and records of all data used to complete the application for this permit, for a period of at least 3 years from the date of this permit, measurement, report or application. This period may be extended by request of the Agency at any time.

(c) Records of monitoring information shall include:

(1) The date, exact place, and time of sampling or measurements;

(2) The individual(s) who performed the sampling or measurements;

(3) The date(s) analyses were performed;

(4) The individual(s) who performed the analyses;

(5) The analytical techniques or methods used; and

(6) The results of such analyses.

(d) Monitoring must be conducted according to test procedures approved under 40 CFR Part 136, unless other test procedures have been specified in this permit. Where no test procedure under 40 CFR Part 136 has been approved, the permittee must submit to the Agency a test method for approval. The permittee shall calibrate and perform maintenance procedures on all monitoring and analytical instrumentation at intervals to ensure accuracy of measurements.

(11) **Signatory requirement.** All applications, reports or information submitted to the Agency shall be signed and certified.

(a) **Application.** All permit applications shall be signed as follows:

(1) **For a corporation:** by a principal executive officer of at least the level of vice president or a person or position having overall responsibility for environmental matters for the corporation;

(2) **For a partnership or sole proprietorship:** by a general partner or the proprietor, respectively; or

(3) **For a municipality, State, Federal, or other public agency:** by either a principal executive officer or ranking elected official.

(b) **Reports.** All reports required by permits, or other information requested by the Agency shall be signed by a person described in paragraph (a) or by a duly authorized representative of that person. A person is a duly authorized representative only if:

(1) The authorization is made in writing by a person described in paragraph (a); and

(2) The authorization specifies either an individual or a position responsible for the overall operation of the facility, from which the discharge originates, such as a plant manager, superintendent or person of equivalent responsibility; and

(3) The written authorization is submitted to the Agency.

(c) **Changes of Authorization.** If an authorization under (b) is no longer accurate because a different individual or position has responsibility for the overall operation of the facility, a new authorization satisfying the requirements of (b) must be submitted to the Agency prior to or together with any reports, information, or applications to be signed by an authorized representative.

(12) **Reporting requirements.**

(a) **Planned changes.** The permittee shall give notice to the Agency as soon as possible of any planned physical alterations or additions to the permitted facility.

(b) **Anticipated noncompliance.** The permittee shall give advance notice to the Agency of any planned changes in the permitted facility or activity which may result in noncompliance with permit requirements.

(c) **Compliance schedules.** Reports of compliance or noncompliance with, or any progress reports on, interim and final requirements contained in any compliance schedule of this permit shall be submitted no later than 14 days following each schedule date.

(d) **Monitoring reports.** Monitoring results shall be reported at the intervals specified elsewhere in this permit.

(1) Monitoring results must be reported on a Discharge Monitoring Report (DMR).

(2) If the permittee monitors any pollutant more frequently than required by the permit, using test procedures approved under 40 CFR 136 or as specified in the permit, the results of this monitoring shall be included in the calculation and reporting of the data submitted in the DMR.

(3) Calculations for all limitations which require averaging of measurements shall utilize an arithmetic mean unless otherwise specified by the Agency in the permit.

(e) **Twenty-four hour reporting.** The permittee shall report any noncompliance which may endanger health or the environment. Any information shall be provided orally within 24 hours from the time the permittee becomes aware of the circumstances. A written submission shall also be provided within 5 days of the time the permittee becomes aware of the circumstances. The written submission shall contain a description of the noncompliance and its cause; the period of noncompliance, including exact dates and time; and if the noncompliance has not been corrected, the anticipated time it is expected to continue; and steps taken or planned to reduce, eliminate, and prevent reoccurrence of the noncompliance. The following shall be included as information which must be reported within 24 hours:

(1) Any unanticipated bypass which exceeds any effluent limitation in the permit;

(2) Violation of a maximum daily discharge limitation for any of the pollutants listed by the Agency in the permit to be reported within 24 hours.

The Agency may waive the written report on a case-by-case basis if the oral report has been received within 24 hours.

(f) **Other noncompliance.** The permittee shall report all instances of noncompliance not reported under paragraphs (12)(c), (d), or (e), at the time monitoring reports are submitted. The reports shall contain the information listed in paragraph (12)(e).

(g) **Other information.** Where the permittee becomes aware that it failed to submit any relevant facts in a permit application, or submitted incorrect information in a permit application, or in any report to the Agency, it shall promptly submit such facts or information.

(13) **Transfer of permits.** A permit may be automatically transferred to a new permittee if:

(a) The current permittee notifies the Agency at least 30 days in advance of the proposed transfer date;

(b) The notice includes a written agreement between the existing and new permittees containing a specific date for transfer of permit responsibility, coverage and liability between the current and new permittees; and

(c) The Agency does not notify the existing permittee and the proposed new permittee of its intent to modify or revoke and reissue the permit. If this notice is not received, the transfer is effective on the date specified in the agreement.

(14) All manufacturing, commercial, mining, and silvicultural dischargers must notify the Agency as soon as they know or have reason to believe:

(a) That any activity has occurred or will occur which would result in the discharge of any toxic pollutant identified under Section 307 of the Clean Water Act which is not limited in the permit, if that discharge will exceed the highest of the following notification levels:

(1) One hundred micrograms per liter (100 ug/l);

(2) Two hundred micrograms per liter (200 ug/l) for acrolein and acrylonitrile; five hundred micrograms per liter (500 ug/l) for 2,4-dinitrophenol and for 2-methyl-4,6 dinitrophenol; and one milligram per liter (1 mg/l) for antimony.

(3) Five (5) times the maximum concentration value reported for that pollutant in the NPDES permit application; or

(4) The level established by the Agency in this permit.

(b) That they have begun or expect to begin to use or manufacture as an intermediate or final product or byproduct any toxic pollutant which was not reported in the NPDES permit application.

(15) All Publicly Owned Treatment Works (POTWs) must provide adequate notice to the Agency of the following:

(a) Any new introduction of pollutants into that POTW from an indirect discharge which would be subject to Sections 301 or 306 of the Clean Water Act if it were directly discharging those pollutants; and

(b) Any substantial change in the volume or character of pollutants being introduced into that POTW by a source introducing pollutants into the POTW at the time of issuance of the permit.

(c) For purposes of this paragraph, adequate notice shall include information on (i) the quality and quantity of effluent introduced into the POTW, and (ii) any anticipated impact of the change on the quantity or quality of effluent to be discharged from the POTW.

(16) If the permit is issued to a publicly owned or publicly regulated treatment works, the permittee shall require any industrial user of such treatment works to comply with federal requirements concerning:

(a) User charges pursuant to Section 204(b) of the Clean Water Act, and applicable regulations appearing in 40 CFR 35;

(b) Toxic pollutant effluent standards and pretreatment standards pursuant to Section 307 of the Clean Water Act; and

(c) Inspection, monitoring and entry pursuant to Section 308 of the Clean Water Act.

(17) If an applicable standard or limitation is promulgated under Section 301(b)(2)(C) and (D), 304(b)(2), or 307(a)(2) and that effluent standard or limitation is more stringent than any effluent limitation in the permit, or controls a pollutant not limited in the permit, the permit shall be promptly modified or revoked, and reissued to conform to that effluent standard or limitation.

(18) Any authorization to construct issued to the permittee pursuant to 35 Ill. Adm. Code 309.154 is hereby incorporated by reference as a condition of this permit.

(19) The permittee shall not make any false statement, representation or certification in any application, record, report, plan or other document submitted to the Agency or the USEPA, or required to be maintained under this permit.

(20) The Clean Water Act provides that any person who violates a permit condition implementing Sections 301, 302, 306, 307, 308, 318, or 405 of the Clean Water Act is subject to a civil penalty not to exceed $10,000 per day of such violation. Any person who willfully or negligently violates permit conditions implementing Sections 301, 302, 306, 307, or 308 of the Clean Water Act is subject to a fine of not less than $2,500 nor more than $25,000 per day of violation, or by imprisonment for not more than one year, or both.

(21) The Clean Water Act provides that any person who falsifies, tampers with, or knowingly renders inaccurate any monitoring device or method required to be maintained under permit shall, upon conviction, be punished by a fine of not more than $10,000 per violation, or by imprisonment for not more than 6 months per violation, or by both.

(22) The Clean Water Act provides that any person who knowingly makes any false statement, representation, or certification in any record or other document submitted or required to be maintained under this permit shall, including monitoring reports or reports of compliance or non-compliance shall, upon conviction, be punished by a fine of not more than $10,000 per violation, or by imprisonment for not more than 6 months per violation, or by both.

(23) Collected screening, slurries, sludges, and other solids shall be disposed of in such a manner as to prevent entry of those wastes (or runoff from the wastes) into waters of the State. The proper authorization for such disposal shall be obtained from the Agency and is incorporated as part hereof by reference.

(24) In case of conflict between these standard conditions and any other condition(s) included in this permit, the other condition(s) shall govern.

(25) The permittee shall comply with, in addition to the requirements of the permit, all applicable provisions of 35 Ill. Adm. Code, Subtitle C, Subtitle D, Subtitle E, and all applicable orders of the Board.

(26) The provisions of this permit are severable, and if any provision of this permit, or the application of any provision of this permit is held invalid, the remaining provisions of this permit shall continue in full force and effect.

(Rev. 3-13-98)

Exhibit B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 5
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

MAR 2 1 2014

REPLY TO THE ATTENTION OF:
WQ-16J

Marcia T. Willhite, Chief
Bureau of Water
Illinois Environmental Protection Agency
P.O. Box 19276
Springfield, Illinois 62794-9276

Dear Ms. Willhite:

The Illinois Environmental Protection Agency (IEPA) forwarded to the U.S. Environmental Protection Agency the variance application submitted by the Sanitary District of Decatur (SDD) to the Illinois Pollution Control Board (IPCB) in IPCB Docket No. 2014-11. IEPA requested that EPA review and comment on the application. This letter provides those comments.

SDD's variance application discusses EPA's March 15, 2013, letter that disapproved Illinois' request for approval of a variance for CITGO Petroleum Corp. under section 303(c) of the Clean Water Act (CWA). EPA explained in the March 15, 2013, letter that, under the CWA and EPA's implementing regulations, a variance can only be approved by EPA as a revision to water quality standards in accordance with section 303(c) of the CWA if, among other things, the state can demonstrate that the designated use for the water body at issue is not attainable for at least one of the reasons specified at 40 CFR 131.10(g). As explained in the Federal Register notice of EPA's recently proposed revisions to EPA's water quality standards regulations that is cited in SDD's variance application, this has been EPA's longstanding interpretation of the CWA and EPA's implementing regulations, which EPA has consistently applied since 1977. *See* 78 Fed. Reg. 54518, 54531 (Sept. 4, 2013). This continues to be EPA's interpretation and nothing in the Federal Register notice or in EPA's proposed revisions to its water quality regulations changes that longstanding interpretation.

Thus, for a variance to be approvable by EPA under the section 303(c) of the CWA and EPA's implementing regulations, Illinois will be required to affirmatively demonstrate that it is not feasible to attain the General Use designation for the Sangamon River for one of the reasons specified at 40 CFR 131.10(g). We urge the IEPA and the IPCB to carefully evaluate SDD's variance request to determine whether this threshold has been met. In doing so, IEPA and IPCB should consider whether all alternatives for reducing the discharge of nickel into the Sangamon River have been evaluated and demonstrated to be infeasible; including, but not limited to, all alternatives for treating discharges from SDD's wastewater treatment plant, all alternatives for reducing nickel in the wastewater from the Archer Daniels Midland (ADM) facility before it enters SDD's sewer system such as treatment alternatives and process changes, and all alternatives for eliminating ADM's discharges into SDD's sewer system such as piping ADM's

discharges away from the sewer system to another receiving stream location where there might be more available dilution than currently exists in the portion of the Sangamon River into which SDD discharges. In addition, IEPA and IPCB should recognize that, as explained in EPA's March 15, 2013, letter disapproving the CITGO variance, the feasibility threshold in 131.10(g) is different from the "arbitrary and unreasonable hardship" threshold set forth at 415 ILCS5/35(a).

We hope that these comments are useful as IEPA and IPCB evaluate whether SDD's variance application is consistent with federal requirements. If you have any questions about these comments, please contact Linda Holst at 312-886-6758 or holst.linda@epa.gov or Robie Anson, at 312-886-1502 or anson.robie@epa.gov of my staff.

Sincerely,

Tinka G. Hyde
Director, Water Division

cc: Tim Kluge, Sanitary District of Decatur

Exhibit C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGIONAL ADMINISTRATOR
REGION 5
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

MAR 15 2013

John M. Kim, Director
Illinois Environmental Protection Agency
1021 North Grand Avenue East
P.O. Box 19276
Springfield, Illinois 62794-9276

Dear Mr. Kim:

On November 15, 2012, the Illinois Environmental Protection Agency (Illinois EPA) transmitted a variance, issued by the Illinois Pollution Control Board (IPCB or the Board) to CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C., for review and approval by the U.S. Environmental Protection Agency in accordance with section 303(c) of the Clean Water Act (CWA). IPCB granted the variance from the total dissolved solids (TDS) criterion in Illinois' water quality standards at 35 Ill. Adm. Code 302.407 for protection of Illinois' indigenous aquatic life designated use for the Chicago Sanitary and Ship Canal (CSSC), a segment of the Chicago Area Waterway System. As described below, EPA disapproves the variance.

IPCB granted the variance in accordance with a state statute that allows the Board to grant regulatory relief when "compliance with any rule or regulation, requirement or order of the Board would impose an arbitrary or unreasonable hardship." The variance effectively removed for a time-limited period the indigenous aquatic life use and removed the TDS criterion necessary to protect that use for that period of time.

The CWA and federal regulations do not allow states to remove designated uses or modify criteria simply because a state believes that such standards "would impose an arbitrary or unreasonable hardship." Instead, under EPA's regulations, a state can only remove a designated use specified in section 101(a)(2) of the CWA, or a subcategory thereof, if, among other things, the state demonstrates that it is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g). Similarly, states can only modify criteria necessary to protect designated uses if the state provides an adequate scientific rationale demonstrating that the revised criteria protect designated uses.

While Illinois EPA asserts that the variance is justified as a time-limited removal of the indigenous aquatic life designated use, Illinois did not provide appropriate technical and scientific data and analyses to support such a use removal as required by 40 CFR 131.5(a)(4).

Specifically, Illinois did not provide appropriate technical and scientific data and analyses demonstrating that the indigenous aquatic life designated use was not attainable for any of the reasons specified at 40 CFR 131.10(g), and so Illinois did not submit "[u]se designations consistent with the provisions of sections 101(a)(2) and 303(c)(2) of the Act" as required by 40 CFR 131.6(a). Consequently, EPA disapproves Illinois' effective time-limited removal of the indigenous aquatic life designated use based upon EPA's conclusion that it was not based upon appropriate technical and scientific data and analyses as required by 40 CFR 131.5(a)(1), 131.5(a)(4), 131.5(a)(5) and 40 CFR 131.10. Furthermore, to the extent that the variance modified Illinois' criteria for protection of the indigenous aquatic life designated use by effectively eliminating the applicable TDS criterion, EPA disapproves the modification in accordance with 40 CFR 131.5(a)(2) and (5) because no adequate scientific rationale demonstrating that removal of the TDS criterion would be protective of the indigenous aquatic life designated use has been provided as required by 40 CFR 131.6(b), (c) and (f) and 131.11(a). The enclosed document, entitled "Basis for EPA's Disapproval of IPCB Decision Granting Variance to CITGO Petroleum Corp. and PDV Midwest Refining, L.L.C.," more fully sets forth the basis for EPA's decision.

To address this disapproval, Illinois needs to take action so that the indigenous aquatic life designated use and the TDS criterion to protect that use at 35 Ill. Adm. Code 302.407 are fully effective under Illinois law with respect to the CSSC, including with respect to discharges into the CSSC from the oil refinery owned by CITGO Petroleum Corporation and PDV Midwest Refining L.L.C.

The impact of today's disapproval is that, for CWA purposes, the indigenous aquatic life designated use and the TDS criterion to protect that use at 35 Ill. Adm. 302.407 apply to the CSSC, including with respect to discharges into the CSSC from the oil refinery owned by CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C., notwithstanding IPCB's variance decision. The use and criterion will apply for CWA purposes until EPA approves a change, deletion, or addition to the water quality standards for the segments impacted by today's disapprovals, or promulgates standards for those segments. *See* 40 CFR 131.21(e).

If Illinois wants to take the effects of deicing activities in the Chicago area into account in the water quality standards for the CSSC, Illinois could attempt to do so as part of IPCB's proceedings pertaining to aquatic life use designations and criteria for the Chicago Area Waterway System in IPCB Subdocket Nos. R2008-09(C) and (D). Specifically, Illinois could perform a structured, scientific assessment of the attainability of aquatic life uses, taking into account deicing activities, and of the criteria necessary to protect aquatic life uses, and revise water quality standards accordingly. Illinois could submit any such revisions to EPA for approval, along with the methods used, analyses conducted, scientific rationale and other information demonstrating the appropriateness under federal law of any revised aquatic life designated use for the CSSC and any new or revised criteria for the protection of the revised aquatic life designated use that differ from those specified at 35 Ill. Adm. Code 302.407.

If you have any questions regarding this matter, please contact me or your staff may contact Linda Holst, Chief, Water Quality Branch, at (312) 886-6758.

Sincerely,

Susan Hedman
Regional Administrator

Enclosure

cc: Marcia Willhite, Illinois EPA
John Therriault, Illinois Pollution Control Board, Clerk's Office

Basis for EPA's Disapproval of Illinois Pollution Control Board's Decision Granting a Variance to CITGO Petroleum Corp. and PDV Midwest Refining, L.L.C."

Date: MAR 15 2013

I. Introduction

On November 15, 2012, the Illinois Environmental Protection Agency (Illinois EPA) submitted a request for the U.S. Environmental Protection Agency to approve in accordance with section 303(c) of the Clean Water Act (CWA), a revision to water quality standards for the Chicago Sanitary and Ship Canal (CSSC). Specifically, Illinois EPA requested that EPA approve an Illinois Pollution Control Board (IPCB) decision granting a "variance" to CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C., from the total dissolved solids (TDS) criterion in Illinois' water quality standards at 35 Ill. Adm. Code 302.407 for protection of Illinois' designated use for aquatic life in the CSSC. *See CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C v. IEPA*, PCB 12-94 (October 18, 2012) (hereinafter "*CITGO Variance Decision*") *available at* http://www.ipcb.state.il.us/documents/dsweb/Get/Document-77765. The IPCB granted the variance in accordance with a state statute that allows IPCB to grant regulatory relief when "compliance with any rule or regulation, requirement or order of the Board would impose an arbitrary or unreasonable hardship." 415 ILCS 5/35(a); *see also CITGO Variance Decision* at 20.

II. Legal Background

A. Designated Uses and Water Quality Criteria

Section 101(a)(2) of the CWA states the national interim goal of achieving by July 1, 1983, "water quality which provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water" (hereafter collectively referred to as "the uses specified in section 101(a)(2)"), wherever attainable. Section 303 of the CWA requires states to adopt water quality standards for waters of the United States within their respective jurisdictions. Section 303(c) of the CWA requires, among other things, that state water quality standards include the designated use or uses to be made of the waters and water quality criteria based upon such uses. Section 303(c)(2)(A) of the CWA requires that water quality standards "protect the public health or welfare, enhance the quality of water and serve the purposes" of the CWA. The EPA's regulations at 40 CFR 131.2 explain that:

> "Serve the purposes of the Act" (as defined in sections 101(a)(2) and 303(c) of the Act) means that water quality standards should, wherever attainable, provide water quality for the protection and propagation of fish, shellfish and wildlife and for recreation in and on the water and take into consideration their use and value of [*sic*] public water supplies, propagation of fish, shellfish, and wildlife, recreation in and on the water, and agricultural, industrial, and other purposes including navigation.

EPA's regulations at 40 CFR Part 131 interpret and implement sections 101(a)(2) and 303(c)(2)(A) of the CWA through a requirement that water quality standards include the uses specified in section 101(a)(2) of the CWA, unless those uses have been shown to be unattainable, in which case a state can adopt subcategories of the uses specified in section 101(a)(2) which require less stringent criteria. *See* 40 CFR 131.5(a)(4), 131.6(a), and 131.10(j), and 131.20(a); *see also Idaho Mining Association v. Browner*, 90 F.Supp. 2d 1078, 1092 (D. Id. 2000); 68 Fed. Reg. 40428, 40430-31 (July 27, 2003). 40 CFR 131.10(g) provides that, once a state designates the uses specified in section 101(a)(2) of the CWA or subcategories thereof for a specific water body, the state can only remove the designated use if, among other things, "the [s]tate can demonstrate that attaining the designated use is not feasible [for at least one of the six reasons set forth at 40 CFR 131.10(g)]."

When a state adopts designated uses that include the uses specified in section 101(a)(2) of the CWA or subcategories thereof, the state must also adopt "water quality criteria that protect the designated use." 40 CFR 131.11(a). "Such criteria must be based on sound scientific rationale and must contain sufficient parameters or constituents to protect the designated use." *Id.* Unlike with designated uses, nothing in the CWA or EPA's regulations allows states to relax or modify criteria, based on concepts of attainability, to levels that are not protective of the designated use. Instead, if criteria are not attainable, the CWA and EPA's regulations allow states to (1) remove the current designated use after demonstrating, among other things, that attaining the current designated use is not feasible for one of the 40 CFR 131.10(g) reasons, and replace it with a subcategory of use and, then, (2) adopt new, potentially less stringent, criteria necessary to protect the new designated use.

B. Variances

EPA has long recognized that, where a state satisfies all of the requirements in 40 CFR Part 131 for removing designated uses (or subcategories of uses), including demonstrating that it is not feasible to attain the designated use for one of the reasons specified at 40 CFR 131.10(g), EPA could also approve a state decision to limit the applicability of the use removal to only a single discharger, while continuing to apply the previous use designation and criteria to other dischargers. Such a state decision, which is often referred to as a "variance," can be approved as being consistent with the requirements of the CWA and 40 CFR Part 131. This is because the state's action in limiting the applicability of an otherwise approvable use removal to a single discharger and to a single pollutant is environmentally preferable and would be more stringent than a full use removal; and states have the right to establish more stringent standards under section 510 of the CWA. *See* 58 FR 20802, 20921-22 (April 16, 1993).

C. Water Quality Standard Submission Requirements and EPA Review Authority

40 CFR 131.6 provides that states must submit, among other things, the following to the EPA for review when they adopt new or revised designated uses and criteria:

> (a) Use designations consistent with the provisions or section 101(a)(2) and 303(c)(2) of the Act.

(b) Methods used and analyses conducted to support water quality standards revisions.
(c) Water quality criteria to protect the designated uses.
. . . .
(f) General information which will aid the Agency in determining the adequacy of the scientific basis of the standards which do not include the uses specified in section 101(a)(2) of the Act as well as information on general policies applicable to State standards which may affect their application and implementation.

40 CFR 131.5(a) provides that, in reviewing new or revised use designations and criteria, the EPA must determine, among other things:

(1) Whether the State has adopted water uses which are consistent with the requirements of the Clean Water Act;
(2) Whether the State has adopted criteria that protect the designated uses;
. . . .
(4) Whether the State standards which do not include the uses specified in section 101(a)(2) of the Act are based upon appropriate technical and scientific data and analyses, and
(5) Whether the State submission meets the requirements included in §131.6 of this part.

40 CFR 131.21(c)(2) provides that new or revised water quality standards that are adopted by states do not become applicable water quality standards for purposes of the CWA until after they have been submitted to and approved by EPA in accordance with section 303(c) of the CWA.

III. Illinois' Water Quality Standards for the CSSC

A. Illinois' Adoption and EPA's Approval of Indigenous Aquatic Life Designated Use and Criteria for the CSSC

As noted above, EPA's regulations at 40 CFR Part 131 interpret and implement sections 101(a)(2) and 303(c)(2)(A) of the CWA through a requirement that water quality standards include the uses specified in section 101(a)(2) of the CWA, unless those uses have been shown to be unattainable for one of the reasons set forth at 40 CFR 131.10(g). When consistent with the requirements of 40 CFR 131.10(g), a state can adopt subcategories of the uses specified in section 101(a)(2) which require less stringent criteria. In 1974, Illinois demonstrated that providing for protection and propagation of fish – *i.e.*, one of the uses specified in section 101(a)(2) of the CWA – was not attainable for several waters in the Chicago area, and so Illinois adopted a subcategory of aquatic life use, referred to as "indigenous aquatic life" that it applied to the CSSC. *See* 35 Ill. Adm. Code 302 Subpart D. Waters designated as indigenous aquatic life waters are supposed to be capable of supporting an indigenous aquatic life limited only by the physical configuration of the body of water, characteristics and origin of the water and the presence of contaminants in amounts that do not exceed the water quality standards listed in Subpart D. 35 Ill. Adm. Code 302.402. Illinois also adopted criteria to protect the indigenous aquatic life designated use, including the total dissolved solids (TDS) criterion of 1,500

milligrams per liter (mg/L) set forth at 35 Ill. Adm. Code 302.407. The indigenous aquatic life use and associated criteria applicable to the CSSC were approved previously by EPA[1]

B. Variances Pertaining to the CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C. oil refinery in Lemont, Illinois

The IPCB first granted to CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C. a variance from the TDS criterion on April 21, 2005. *See CITGO Variance Decision* at 3. The variance effectively eliminated the applicability of the TDS criterion of 1,500 mg/L for purposes of deriving a water quality based effluent limit (WQBEL) for TDS in CITGO's National Pollutant Discharge Elimination System permit. The IPCB extended the variance on May 15, 2008, *id.*, and again on October 18, 2012, *id.* at 20. Illinois did not submit either the IPCB's original 2005 variance decision or 2008 extension decision to EPA for review and approval under section 303(c) of the CWA. Consequently, the original 2005 variance and the 2008 extension have never been applicable water quality standards for purposes of the CWA. *See* 40 CFR 131.21(c)(2). On November 15, 2012, Illinois EPA submitted IPCB's October 18, 2012, variance decision to EPA for approval in accordance with section 303(c) of the CWA.

The basis for the variance decision in each instance was IPCB's conclusion that compliance with a WQBEL derived from the TDS criterion "would impose an arbitrary or unreasonable hardship." The variance effectively removed for a time-limited period the indigenous aquatic life designated use and removed the TDS criterion necessary to protect that use for that period of time. Despite statements by Illinois EPA and IPCB that the variances are consistent with federal law (*see* CITGO variance at 17), nothing in the CWA or EPA's water quality standards regulations allows states to remove designated uses or modify criteria on this "hardship" basis alone. Instead, as described above, water quality standards can be revised where it can be demonstrated that it is not feasible to attain a designated use for one of the reasons specified at 40 CFR 131.10(g) (and other requirements are also met); or where criteria are revised based on sound scientific rationale and are protective of applicable designated uses in accordance with 40 CRF 131.6(c) and 131.11(a). As described below, there is no indication in IPCB's 2005, 2008 or 2012 decisions that, in granting and extending the variance, IPCB ever evaluated the feasibility of attaining the indigenous aquatic life use designation in the CSSC utilizing any of the factors in 40 CFR 131.10(g). There also is no indication in IPCB's decisions that removal of the TDS criterion is based upon a sound scientific rationale demonstrating that the indigenous aquatic life designated use would be protected.

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[1] EPA first approved the indigenous aquatic life use applied to the CSSC in 1974 and the adoption of the applicable TDS standard in 1979. In 2011, Illinois revised aspects of its water quality standards pertaining to the Chicago Area Waterway System to update certain designated recreational uses. The revisions also impacted some aspects of the indigenous aquatic life designated use and criteria. On May 16, 2012, EPA approved portions of those revisions and disapproved others. Illinois' 2011 revisions, and EPA's May 16, 2012, action, did not result in any substantive change to either the indigenous aquatic life designated use for the CSSC or the criteria for protection of that use at 35 Ill. Adm. Code 302.407. *See* EPA's May 16, 2012, letter and supporting documents, *available at* http://www.epa.gov/region5/chicagoriver.

IV. EPA's Action on Illinois' Revised Water Quality Standard for the CSSC

A. "Arbitary and Unreasonable Hardship"

EPA cannot approve the IPCB's decision granting the variance as a change to water quality standards solely because the state believes that such standards "would impose an arbitrary or unreasonable hardship." Instead, EPA evaluated Illinois EPA's November 15, 2012 submission to determine whether the change to the standards is consistent with the CWA and federal regulations regarding time-limited use removals (often referred to as "variances to water quality standards") and water quality criteria[2].

B. Time-Limited Use Removal

Illinois EPA, in its November 15, 2012, submission to EPA, asserts that IPCB's variance decision can be justified under 40 CFR 131.10(g)(3) and (g)(6) as a time-limited use removal. Each of these assertions is evaluated below.

1. 40 CFR 131.10(g)(3)

40 CFR 131.10(g)(3) provides that designated uses can be removed "if the [s]tate can demonstrate that attaining the designated use is not feasible because . . . [h]uman caused conditions or sources of pollution prevent the attainment of the use and cannot be remedied or would cause more environmental damage to correct than to leave in place."

As a threshold matter, to justify removing a designated use under 40 CFR 131.10(g)(3), a state must identify with some specificity the "human caused conditions or sources of pollution [that] prevent the attainment of the use." While the record before IPCB is replete with generalized assertions that winter de-icing activities using road salt and other compounds cause TDS levels in the CSSC to exceed the TDS criterion, there is nothing in the state record that adequately identifies with any specificity where these activities are taking place, what entities are responsible for these activities, and what amount of the total TDS load into the CSSC each entity is responsible for.[3] In addition, it is unclear from the record and IEPA's November 15, 2012,

[2] EPA also evaluated Illinois EPA's subsequent submission of more detailed references to documents and information Illinois EPA believed to be relevant to the review of the CITGO variance (email from S. Sofat to L. Holst, dated 2/4/13).

[3] Specifically, a state should develop and evaluate information on the amount of loadings of the pollutant at issue from each source (including any point source that is the subject of a variance request) relative to the other sources and also relative to the total loadings to the water body. Here, although there was testimony in the state administrative record that, during snowmelt, the oil refinery effluent makes up between 0.6 to 1% of the total TDS load in the CSSC (Huff 2005 testimony at 35-36), there is no similar information in the record on the other specific sources of TDS. Information on the relative loadings from each source is important in evaluating potential remedial measures.

submission to EPA whether, and to what extent, the state believes that TDS discharges from the oil refinery are one of the "sources" that prevent attainment of the designated use. In sum, Illinois has not adequately identified the "human caused conditions or sources of pollution [that] prevent the attainment of the use."

Once a state identifies with specificity the "human caused conditions or sources of pollution [that] prevent the attainment of the use," then, to justify removing a designated use under 40 CFR 131.10(g)(3), the state must also demonstrate either that the conditions or sources "cannot be remedied" or that implementation of the remedy "would cause more environmental damage to correct than to leave in place." One way that states can make such a demonstration would be to present information on the cost and technical feasibility of a reasonable range of potential remedial measures that could be implemented so that those "conditions or sources of pollution" no longer prevent the attainment of the use. The state must then demonstrate either that it is not feasible to implement such remedial measures (thereby demonstrating that the "human caused conditions or sources of pollution cannot be remedied") or that implementation of such remedial measures would "cause more environmental damage to correct than to leave in place." Here, the state administrative record only includes information regarding the cost, technical feasibility and environmental impacts of remedial measures for one of the sources of pollution – the oil refinery – into the CSSC. The state has not identified – much less evaluated the costs, technical feasibility and environmental impact of – remedial measures for the other sources that the state asserts prevent attainment of the use: *i.e.*, the sources responsible for winter de-icing activities.[4] Nor has Illinois demonstrated in any other way that the "human caused conditions or sources of pollution" cannot be remedied or that implementation of such a remedy "would cause more environmental damage to correct than to leave in place."

Because Illinois has not provided sufficient information identifying the "human caused conditions or sources of pollution prevent[ing] attainment of the use," and has not provided sufficient information demonstrating that such human caused conditions or sources of pollution "cannot be remedied or would cause more environmental damage to correct than to leave in place," Illinois has not demonstrated that attaining the designated indigenous aquatic life use is not feasible under 40 CFR 131.10(g)(3).

[4] CITGO appended testimony to its variance request that was presented in a separate rulemaking effort before IPCB in IPCB Docket No. R2008-09(C) regarding the attainability of proposed revisions to the aquatic life use designation and associated chloride criteria that IPCB is considering adopting for the CSSC. Specifically, CITGO appended testimony that "[a]ttainment of chloride criteria [being considered as being necessary to protect the revised aquatic life use designation being considered by IPCB] requires a 50% reduction of deicing salt use," and that attainable reduction goals could be up to 30%, citing one municipality. However, no such information or analysis is given for the TDS, the pollutant at issue here.

2. 40 CFR 131.10(g)(6)

In regards to 40 CFR 131.10(g)(6), Illinois did provide limited information regarding the costs of one alternative for reducing TDS discharges from the oil refinery using evaporation technology. However, there is nothing in the record providing an evaluation or a demonstration of how implementation of this control or any other controls more stringent than those required by sections 301(b) and 306 of the CWA to control TDS would result in "substantial and widespread economic and social impact." Consequently, Illinois has not adequately demonstrated "that attaining the designated [indigenous aquatic life] use is not feasible because . . . [c]ontrols more stringent than those required by sections 301(b) and 306 of the [CWA] would result in substantial and widespread economic and social impact." 40 CFR 131.10(g)(6).

C. Criteria Revision

Illinois EPA also notes in its November 15, 2012, submission that (1) IPCB removed the TDS criterion for Illinois General Use waters in 2008 and (2) Illinois is considering removing the TDS criterion applicable to the CSSC in the context of adopting revised aquatic life use designations and associated criteria in the Chicago Area Waterway System proceedings, in IPCB Docket No. R2008-09.[5] However, Illinois EPA has not asserted, and the IPCB's orders do not suggest, that IPCB's variance decision can be justified as a revision to the criteria for protection of the indigenous aquatic life designated use for the CSSC. Even if Illinois EPA had made such an assertion, IPCB's variance decision would not be approvable as a modification to criteria. This is because, as described below, the administrative record for the variance decision lacks sufficient scientific rationale as required by 40 CFR 131.6(b), (c) and (f) and 131.11(a) as to why removal of the TDS criterion would be protective of the current indigenous aquatic life use.

The scientific rationale as to why IPCB's removal of the TDS criterion was protective of the aquatic life uses in General Use waters is that (1) chlorides and sulfates are constituents of TDS; (2) IPCB adopted chloride and sulfate criteria for the General Use waters, and so (3) there is no longer any need to include the TDS criterion as a surrogate parameter for chlorides and sulfates. *See* IPCB's First Opinion and Order in "Triennial Review of Sulfate and Total Dissolved Solids Water Quality Standards," Docket No. R07-09 (September 20, 2007), at 26, *available at* http://www.ipcb.state.il.us/documents/dsweb/Get/Document-58772. Illinois EPA's proposal to not include TDS criterion for any aquatic life use designations that are ultimately adopted for the Chicago Area Waterway System relies on the same scientific rationale. *See* IEPA's Statement of Reasons at 78-79, filed by IEPA on October 26, 2007, in IPCB Docket No. R2008-09, *available at* http://www.ipcb.state.il.us/documents/dsweb/Get/Document-59147. IPCB's variance decision does not include adoption of chloride and sulfate criteria and so is not supported by either the scientific rationale underlying removal of the TDS criterion from the General Use water quality

[5] Illinois EPA's proposal to remove the TDS criterion can be found in IPCB's Docket No. R2008-09. After IEPA initiated those proceedings, Docket No. R2008-09 was broken into four subdockets. Subdocket No. R2008-09(C) pertains to aquatic life use designations for the Chicago Area Water System, including the CSSC. Subdocket No. R2008-09(D) pertains to criteria necessary to protect any revised aquatic life designations.

standards or Illinois EPA's rationale to remove the TDS criterion from future aquatic life use designations for the Chicago Area Waterway System.

There is opinion evidence in the state administrative record from 2005 indicating that incremental increases in TDS levels in the CSSC resulting from operation of an air pollution control wet gas scrubber at the refinery would have no impact on the receiving stream. *See* PCB 05-85 Opinion and Order, April 25, 2005 at 13. The basis for that opinion appears to be evidence presented by the petitioners that (1) even with the incremental TDS increases, the TDS levels outside of the mixing zone in the CSSC during most times of the year would still be substantially below the 1,500 mg/l TDS criterion, and (2) in the rare instances where deicing activities cause TDS levels in the CSSC to exceed 1,500 mg/l at the refinery's discharge point, the incremental increases in the in-stream TDS levels are so small that there is no further adverse impact beyond any adverse impacts resulting from the fact that the TDS levels already exceed 1,500 mg/l. However, nothing in that testimony addresses the question of whether there is a sound scientific rationale for removing the TDS criterion when chloride and sulfate criteria do not replace the existing TDS criterion.

D. Summary of EPA's action to disapprove the CITGO variance

IPCB's variance effectively removed for a time-limited period the indigenous aquatic life designated use and effectively removed the TDS criterion necessary to protect that use for that period of time. EPA disapproves Illinois' variance based upon EPA's conclusion that it was not based upon appropriate technical and scientific data and analyses as required by 40 CFR 131.5(a)(1), 131.5(a)(4), 131.5(a)(5) and 40 CFR 131.10. Furthermore, to the extent that the variance modified Illinois' criteria for protection of the indigenous aquatic life designated use by effectively eliminating the applicable TDS criterion, EPA disapproves the modification in accordance with 40 CFR 131.5(a)(2) and (5) because no adequate scientific rationale demonstrating that removal of the TDS criterion would be protective of the indigenous aquatic life designated use has been provided as required by 40 CFR 131.6(b), (c) and (f) and 131.11(a).

E. Effect of EPA's Action on Endangered and Threatened Species

EPA is disapproving the IPCB's variance decision as explained in this document. This disapproval does not cause any change to Illinois' federally-applicable water quality standards under the CWA. Because there is no change to the State's federally-applicable water quality standards, there is no effect on listed species or their designated habitat. Therefore, Endangered Species Act consultation is not required.

F. Tribal Consultation

On May 4, 2011, EPA issued the "EPA Policy on Consultation and Coordination with Indian Tribes" to address Executive Order 13175, "Consultation and Coordination with Indian Tribal Governments." The EPA Tribal Consultation Policy states that "EPA's policy is to consult on a government-to-government basis with federally recognized tribes when EPA actions and decisions may affect tribal interests."

There are no federally recognized tribes located in the vicinity of the CITGO Petroleum Corporation and PDV Midwest Refining, L.L.C. discharge or downstream within the action area. Therefore, EPA is not engaging in tribal consultation for this action.

Group
Exhibit D

Terranova, Sara

From: Elizabeth Hood <eahood788@gmail.com>
Sent: Wednesday, April 02, 2014 3:55 PM
To: Terranova, Sara
Subject: RE: ipcb
Attachments: 66hnotes.pdf; 141_Sol'yRules&IonCharges.pdf; Complex formation, acid dissociation and solubility product constants.pdf; complexstability.pdf; nickel2005 WHO drinking water note on Nickel.pdf; Nickel Toxicological Overview phe v1.pdf; Metal tolerance in e. coli.pdf; PNAS-1997-Rensing-14326-31 ecoli and zinc and nickel.pdf; nickel toxic.doc.pdf; nickel toxic health.doc.pdf; 437215642.pdf; J. Nutr.-2007-Wright-2809-13.pdf; 1-s2.0-S0304386X10000848-main recovery of precious metals from biological sources.pdf

Ms. Terranova,

I actually was in the middle of writing the previous email when accidentally hit send.

Although I would have liked to review the statements made in the prior email, and add a few additional items.

I can attach citations corresponding to academic validity of the aforementioned statements I made and expand upon it if you would like. I also never got to the human health risks I am concerned about. I will submitt additional comments; hopefully via a word document, regarding human health concerns specifically and specific disease manifestations/specific mechanisms and biomarkers of metal induced pathogenesis.

I would also like to note or briefly comment on the previous and current industrial and/or military munitions activities in the area; and their implications in disease particularly relavent to the exacerbation of known human health effects of Nickel particularly. Although nickel is the substance in review, the toxicity of nickel is dependent on the composition and physiochemical properties of substances also present in the air, water, and soil; as well as accumulative amounts.

Additionally, I have DNR, USGS, USACoE Louisville District, ATSDR, DOE, topographical maps, satillite/aerial imagery, academic literature on sewage waste water discharge, effects of using urban/municiple sewage wastes sludge as compost/fertilizer for crops/ agricultural use, emergence of and spread of drug resistance genes in bacterial species (to be correlated with bacterial species metal ion uptake and/or resillience to metals, and other sourced documents/references of interest.

I have attached several documents including those related to solubility, strength of the coordination complex, other charecteristics of inorganic compounds/complexes, documents related to the toxicity of Ni, WHO recommendations for Ni in water, and adaptations of bacterial species such as e.coli in terms of resillience/uptake/responses to trace metals and multi-drug resistance. DMH's cancer statistics, toxicological epigenetics information, and other additional health information will be included in an additional email to follow this communication.

The concern here is the total exposure and accumulated exposure to all the toxins of the area. Elevated nickel discharge from the wastes of the municiple water system is not the only source of nickel in the area; air, food (as mentioned in the petitioner's original petition for variance), water, and likely applicable to most of the population in the area occupational, and if residing near creeks, streams, the river, and lake one could also add

their home to possible methods of exposure to nickel considering much waste ends up in those water systems and if the conditions are optimal, nickel carbonyl in the air around/ surrounding water carrying nickel species.

The real issue at hand is when is enough enough or too much. The lack of compliance of environmental regulations by upstream industry such as Tate & Lyle and ADM as mentioned by petitioners in 2009 variance request, definitely make the ability of the Sanitary District of Decatur to comply with environmental protection policies much more difficult; however, the health and well-being of entire community particularly by maintaining and securing clean air and clean drinking water is or should be considered a human right; especially considering the majority of the population being adversely affected has no knoweledge of and/or comprehension as to the level of disease in the area that likely is attributed to the air, water, food, place of employment/occupation, and within their homes. The levels of mutagens and hazardous species in the water likely helped ADM executives choose to move their headquarters. If I were them and had repetitive EPA violation paperwork on my desk and large departments of chemists working under me, I wouldn't drink the water or choose to live too close nearby either. Unlike ADM and Tate & Lyle upper management, most of the residents within the area in question do not have such luxury to drink and bathe in bottled water or move/ relocate away from these hazards. 60% of the population (that still have jobs) work in industrial facilities/possitions. Area schools are failing because of poor student performance on Nationalized examinations leaving students and graduates ill prepared and or less attractive to employers or post-secondary education. Nickel and other area pollutants have been known to cause neurological defect including a plethora of learning disabilities, not to mention cancers, autoimmune diseases, bone deformities, skin deformities, lung pathologies, dementia, and neurodegenerative diseases.

Ultimately, the residents and supporters of the industrial presence in the Decatur area have sarificed so much for companies and this nation. If by maintaining nickel emissions only by way of waste water discharge to within levels below regulatory limits, it is that much less exposure to residents that would have been present otherwise.

Rual areas where private wells are the main source of drinking water will likely benefit greatly from reduced contamination. Many sites between the SDoD and Springfield have been identified as being contaminated by toxic substances. Most of which include metals and organic compounds known as PAHs in addition to well known halogenated hydrocarbons and benzenes, have noted/ observed contamination all the way down to ground water. During drought conditions, high levels of nickel discharged into an almost dry Sangamon River bed, has the potential to be even more hazardous in terms of metal associations with toxic hydrocarbon species particularly aromatic hydrocarbons. Greater association of metal ions with aromatic organics leads to enhanced bioaccululation in hydrophobic tissues/regions as well as enhanced access to DNA inside cellular nucleus. The winds are often from the west, therefore any discharged nickel interacting with species causing volatile or gaseous species of nickel to form downstream, would likely be blown back towards the city of Decatur to the east.

It is time to take action in the defense of human rights. Residents should be notified or made aware of the hazards they have encountered in the past and continue to encounter, and continued actions need to be taken to prevent exposure of residents to further regulatory violation concentrations of hazardous substances. This is the air going in our lungs and the water we drink, use for crop irrigation, or recreation.

All for now, more to come.

Sincerely,

Elizabeth Hood

On Apr 2, 2014 10:59 AM, "Terranova, Sara" <Sara.Terranova@illinois.gov> wrote:

Ms. Hood,

Thank you for the questions. I have forwarded your questions and comments to our technical staff in the Bureau of Water. At this point, they will review your comments/questions as they finalize their investigation of the Extension of Variance Petition. Pursuant to 35 Il Adm. Code 104.216(b)(1), the Agency will then provide a summary of your views and the issues raised into our Recommendation to the Illinois Pollution Control Board. We will also attach your comments with our Recommendation for the Board's review as well. If you have any additional questions or comments regarding the technical aspect or the procedural process of this Extension of Variance Petition please just let me know!

Sara G. Terranova

Assistant Counsel

Division of Legal Counsel

Illinois Environmental Protection Agency

1021 North Grand Avenue East, P.O. Box 19276

Springfield, Il 62794-9276

Phone: 217-782-5544 / Fax: 217-782-9807

Sara.Terranova@illinois.gov

From: Elizabeth Hood [mailto:eahood788@gmail.com]
Sent: Wednesday, April 02, 2014 9:29 AM
To: Terranova, Sara
Subject: Re: ipcb

Ms. Terranova,

The top priority question for today:

I have been looking over the variance petition from 2009; does the current variance petition include new nickel and zinc toxicology data (mentioned in timeline of improvements for the future "by 2012/2014" section)? How about new analytical methods data that is more recent than ten years ago plus?

Does the new petition also cite ADM and Tate & Lyle emission of zinc and nickel as a reason they should not be held responsible or be required to take further steps to achieve compliance with set CWA and CAA regulation?

Also, yes, concentration does depend on the amount of water present as well as the amount of nickel and zinc.

At what point is total zinc and nickel water (and air for that matter) content looked at from a chronic exposure/ life time accumulative aspect?

I have many more concerns over the health of the areal population especially in terms of chronic/lifetime exposure; as well as questions about the concentrations of nickel and zinc that can/are being emitted at a single time. Large single emissions can largely go unnoticed or unreported due to the ability of said parties to emitting ridiculously high amounts all in one day, and emit nearly nothing for the remaining part of the four day period to achieve four day average concentrations within "limits" or appear to be of less obvious neglect of emission control regulations.

What are current max concentrations measured in the water for zinc and nickel in the last several years; in ONE sample?

Are atomic absorption methods as well as pre-instrumental analysis separatory methods being used to prevent interferences of other metal species when performing zinc and nickel concentration determination assays?

I can look into this as well on my own, however, how much total zinc and nickel would one consume over a year assuming that human consumption of water daily is eight eight ounce glasses per person per day?

WHO has published recommendations on the amounts of nickel in drinking water that become of health concern in terms of milligrams per kilogram body weight (relatively older citation) and their are more recent sources that also provide suggested concentration ranges that are known to be toxic.

What are the current or average carbon monoxide levels release into the air in the Decatur area? What are current or average carbonate concentrations in the water? Nickel carbonyl is a volatile compound at room temperature and pressure. What is to say that nickel and zinc sludge deposits and emitted nickel ions are not partially complexing with carbonyl (carbon monoxide) ligands and forming toxic nickel carbonyl levels in the air?

Have any coliform bacteria obtained from sampling been characterized genetically or phenotypically (based on metabolic/biochemical properties)? Were pathogenic strains of *Escherichia coli* and other food-born or water-born pathogenic microorganisms identified resulting from fecal coliform bacterial testing? Were mutation rates, virulence, antibiotic resistance, and metal ion toxicity resistance examined, if so what were the outcomes of such assays?

A line from "The Informant" movie comes to mind and inspires me to pose this question: in the film Matt Damon mentions that they used bacteriophages to contaminate the genetically modified e.coli (cloned in inducible gene(s) for the amino acid lysine (according to the movie)) used for lysine production in order to price fix the international lysine prices.

The most common bacteriophage is T4; it is used to transfect e.coli cells as part of the cloning process. Bacteriophages are simply viruses that attack bacteria. T4 has been reported to be able to contain the entire genome coding for Hepatitis B virus; an additional twist to the concern. T4 can aid in the rapid transfer and fragment integrations of DNA that codes for resistance or virulence genes and horizontal transfer. With that said, high concentrations of metal ions like zinc and nickel that now are mixed with sewage discharge with now resistant resilient fecal coliform bacterial species that have been growing on solid nickel and zinc sludge; is used to fertilize farm fields. The Petitioner claims that ADM and the amount of soy beans and corn being

processed there are a major contributors to the source of the zinc and nickel levels later seen in the water. This along with the catalytic waste amounts ultimately ending up in the area's ecosystem.

The main source of water for the surrounding farm ground that grows corn and soybeans is the Sangamon River and it's tributaries, and precipitation that is usually formed when the wind is from the south east high in moisture from the gulf of mexico and encounters dryer cooler air from the west, north west, or south west. The number of particles in the air also aids to their final or more permenant resting place invading water systems or biological systems after uptake from rainfall. Now, additionally, the petitioner puts fecal waste full of stressed or resillient fecal coliform bacteria, that likely show high levels of Ni and Zn cellular uptake, and used them to fertilize farm fields of soy beans and corn. Some if not most of the area's corn is predominantly used for animal feed sources as well as in processed products. I would think that high levels of nickel and zinc as well as likely other trace metals unmentioned in this circumstance found in said corn and soy beans that are processed in the ADM facility, acquire all of the said Ni and Zn from water or soil derived sources.

On Tue, Apr 1, 2014 at 9:56 AM, Terranova, Sara <Sara.Terranova@illinois.gov> wrote:

http://www.ipcb.state.il.us/COOL/external/cases.aspx

PCB 2009-125

PCB 2014-111

Sara G. Terranova

Assistant Counsel

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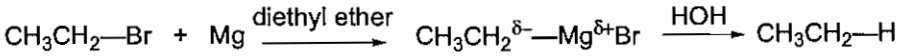
Sara.Terranova@illinois.gov

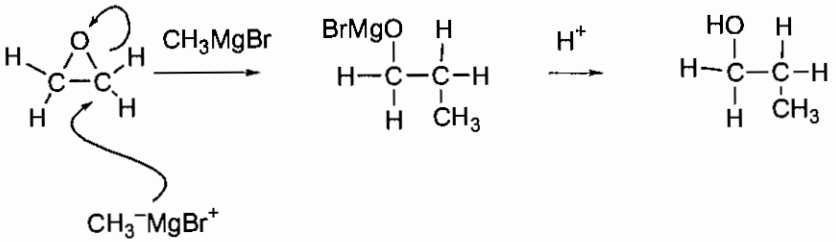
Grignard Reagents

Grignard reagents are organometallic reagents derived from an alkyl halide and magnesium

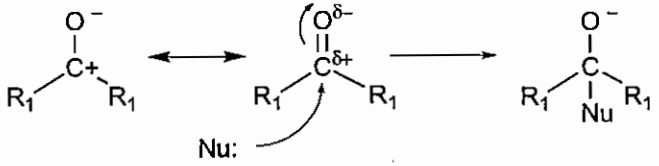
Since the carbon carries a partial negative charge, the carbon is a strong base and a good nucleophile.

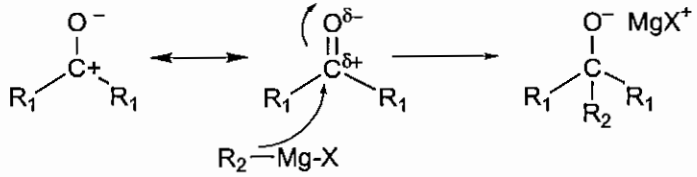


Reaction with epoxides



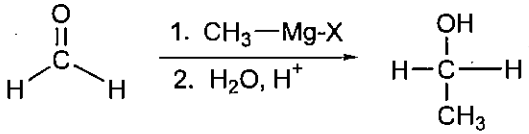
Because carbonyl pi bonds are polarized, they can undergo a reaction called nucleophilic addition: the addition of a nucleophile to an electron deficient pi bond.



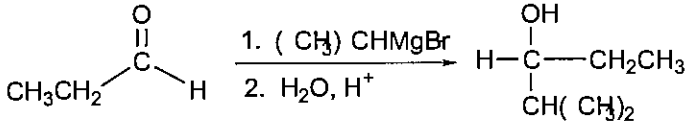


A Grignard reaction with

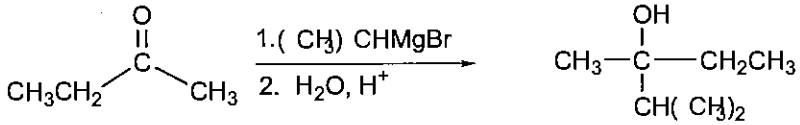
1. formaldehyde produces a primary alcohol



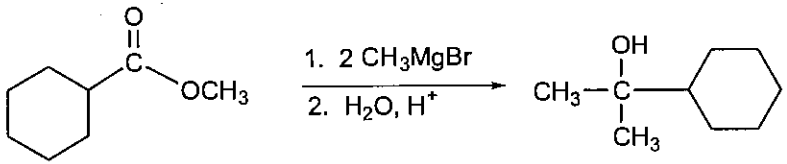
2. an aldehyde produces a secondary alcohol



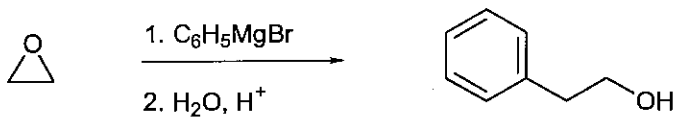
3. a ketone produces a tertiary alcohol

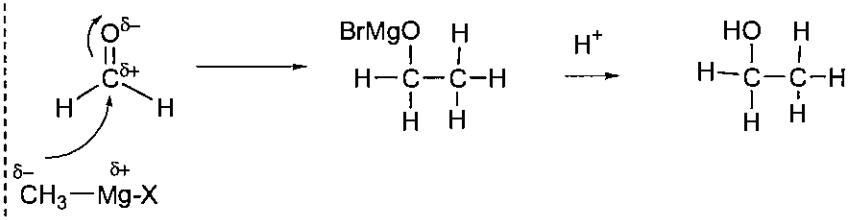


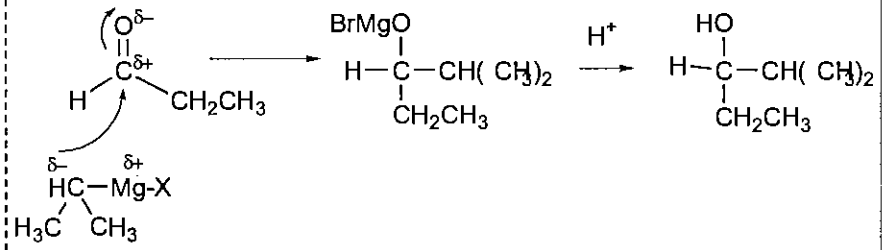
4. an ester produces a tertiary alcohol (addition of two molecules of Grignard reagent)

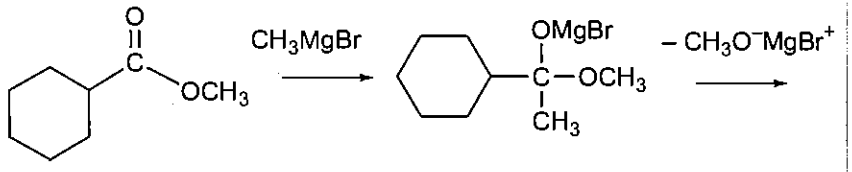


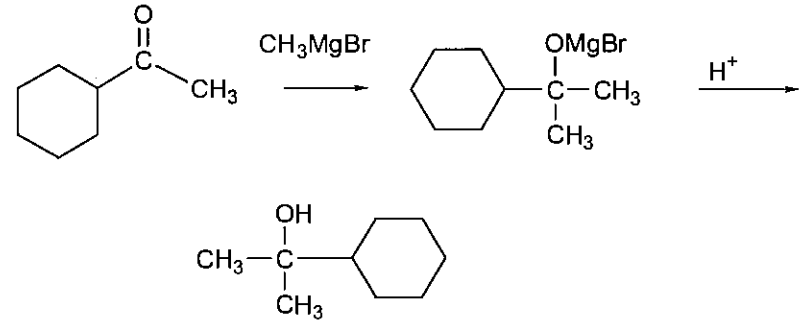
5. ethylene oxide produces a primary alcohol





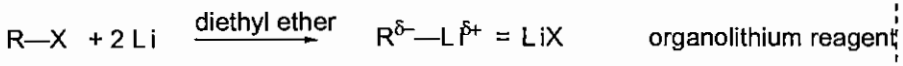




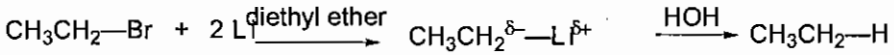


Organolithium Reagents

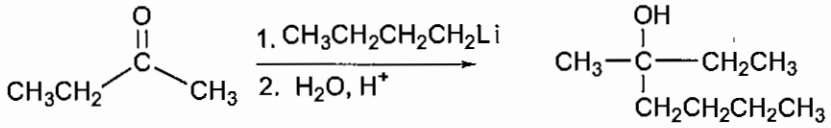
Organolithium reagents are organometallic reagents derived from an alkyl halide and lithium metal



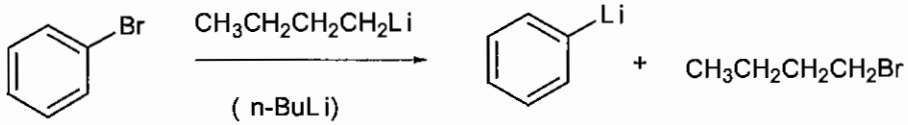
Since the carbon carries a partial negative charge, the carbon is a strong base and a good nucleophile.

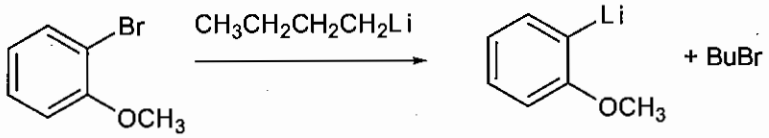


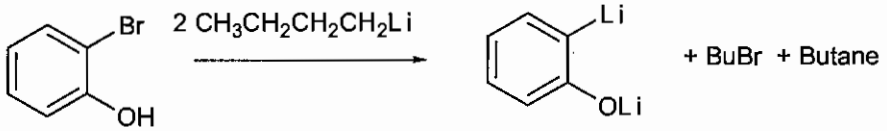
n-Butyl Lithium is a common commercially available organolithium reagent which is used primarily as a strong organic base. It also acts as a nucleophile to add to carbonyl compunds, much like a Grignard reagent.

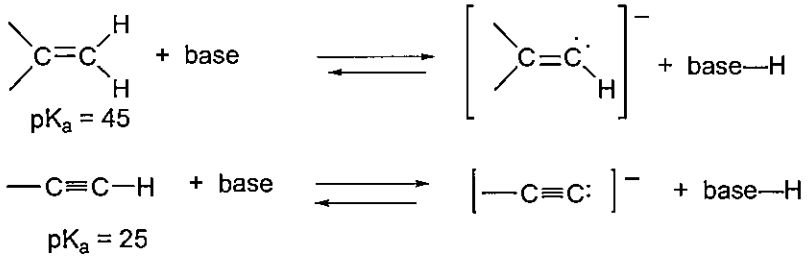


n-Butyl Lithium can also be used to generate aryl and vinyl lthium reagents by lithium-halogen exchange



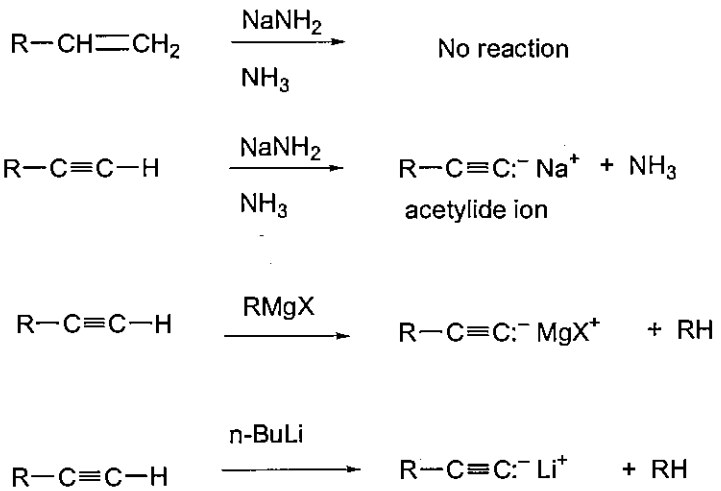




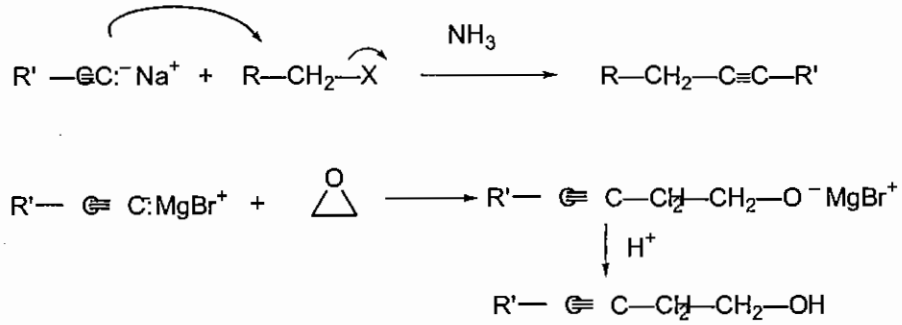


The relatively high acidity of the alkyne —C≡C—H bond is associated with the large degree of s character in the sp C—H bond (50% compared with 33% in sp^2 bonds). The carbon atom is more electronegative in the sp state; thus the C—H bond is more acidic.

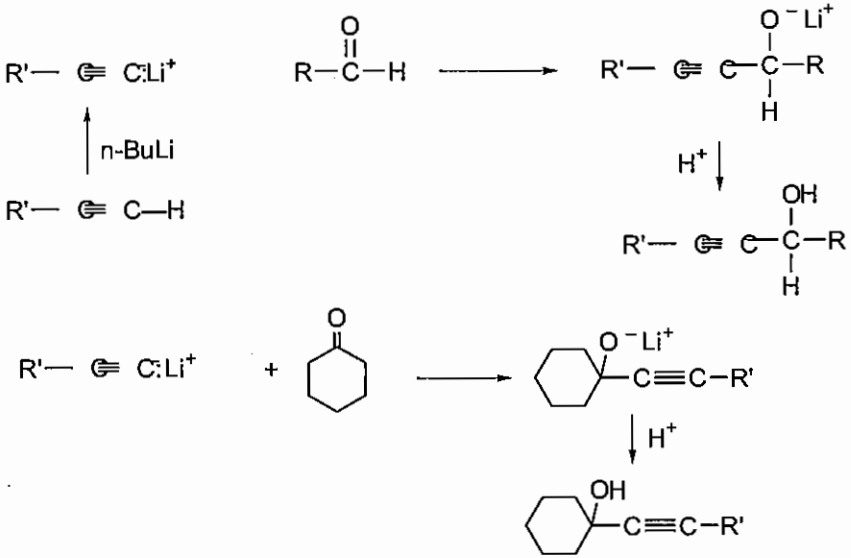
The acetylide ion may be formed by such strong bases as —:NH_2 (pKa 33), RMgX or RLi (pKa 45-50).



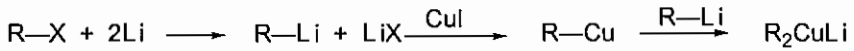
S_N2 reaction with acetylide ion



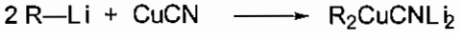
Nucleophilic addition reaction with acetylide ion.



Preparation:
Organocopper reagents can be prepared from organo lithium reagents and Grignard reagents
Cuprates: Me_2CuLi, Bu_2CuLi for common readily available organolithium reagents.

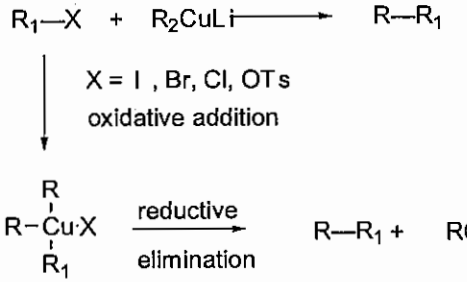


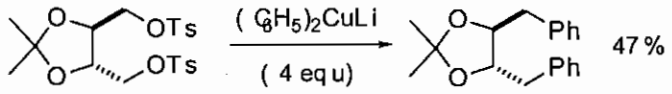
Higher Order Cuprates: somewhat more stable than dialkylcuprates

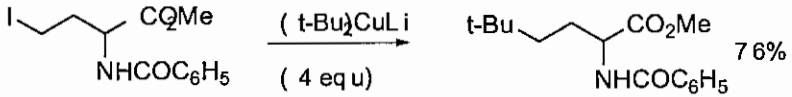


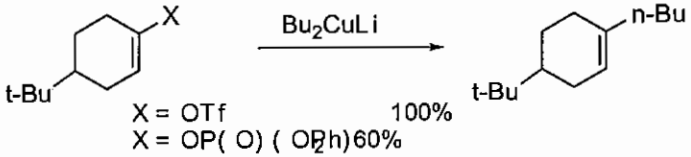
Substitution

Organocopper reagents react with alkyl halides, epoxides, allylic halides, propargylic halides, vinyl halides to give substitution products

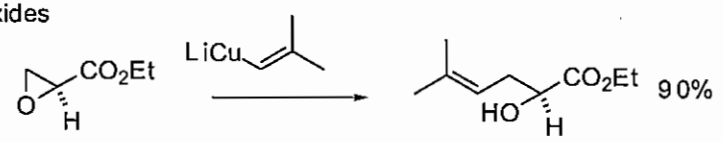


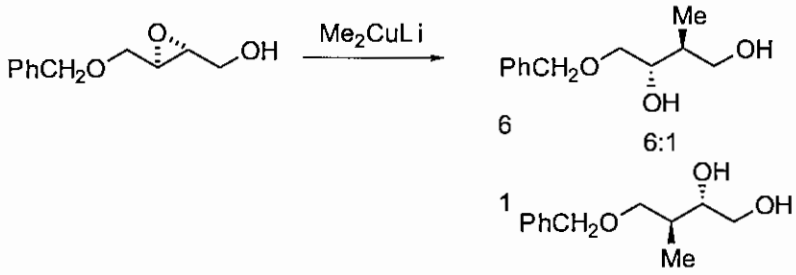


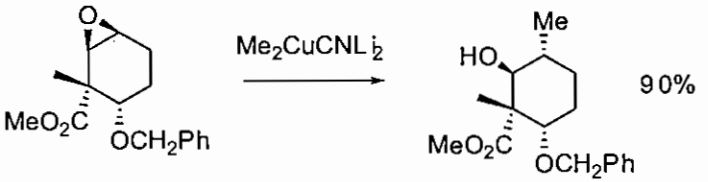




Epoxides







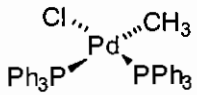
Organometallic complexes contain a metal and coordinated ligands.

The type and number of ligands will depend on the metal and its oxidation state.

Typical ligands of organometallic complexes

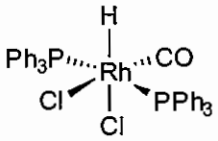
Ligand	Charge	No. of electrons donated
H–	- 1	2
Cl–, Br–, I –	- 1	2
R–, Ar–	- 1	2
	0	2
R_3P:	0	2
:O≡C:	0	2
	- 1	6

Oxidation state of metal is the difference between the overall charge on the complex and the sum of the charges for each ligand.



Cl: -1
CH_3: -1
Ph_3P: 0

Pd: +2

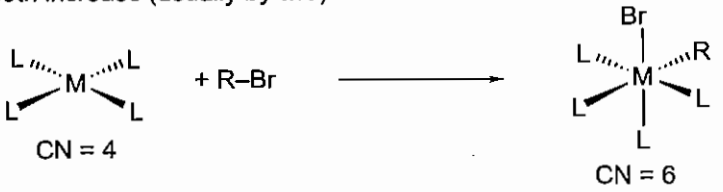


Ph_3P: 0
H: -1
CO: 0
Cl: -1 (X2)
Rh: +3

Organometallic complexes undergo three basic reactions

Oxidative addition
Migratory insertion
reductive elimination

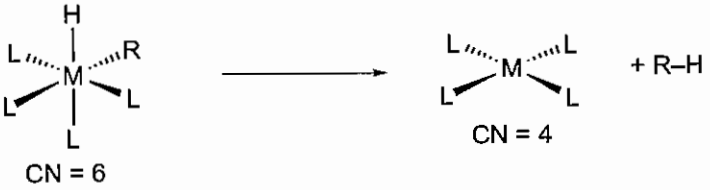
Oxidative addition
The oxidation state and the coordnation number of the metal ion both increase (usually by two)



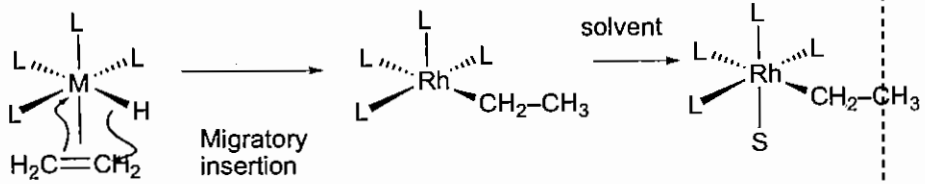
L = generic ligand

reductive elimination

The oxidation state and the coordnation number of the metal ion both decrease (usually by two)

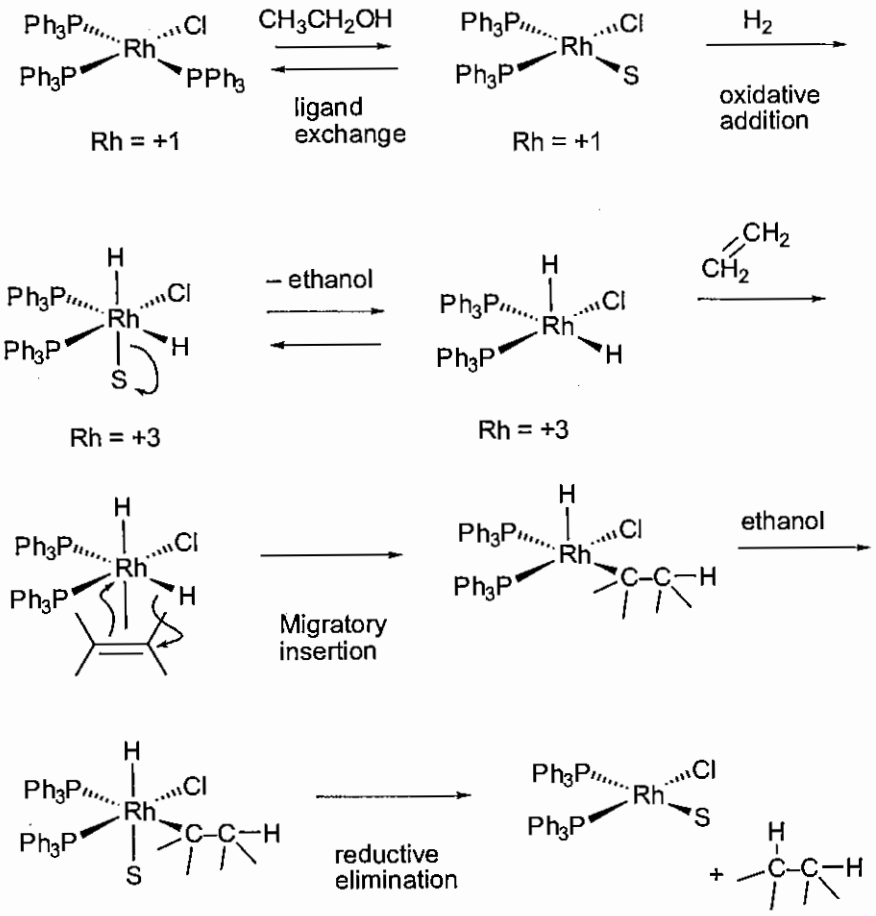


Migratory insertion

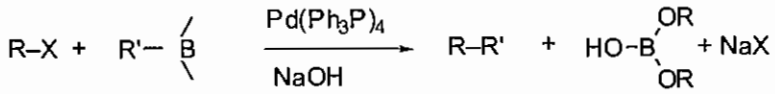


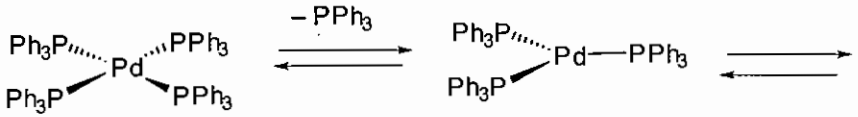
no change in the metal ion oxidation state

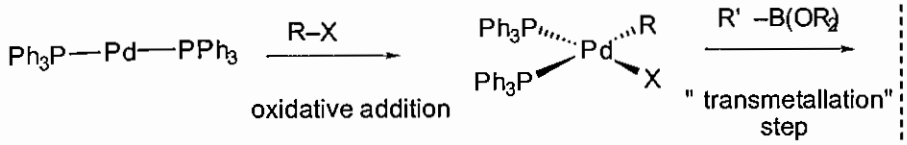
Wilkinson' s Catalyst, $(Ph_3P)_3RhCl$ functions as a catalyst in the presence of hydrogen to convert alkenes into alkanes, ie. hydrogenation.

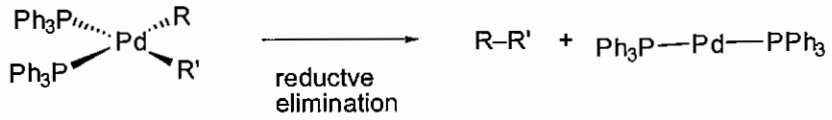


Suz uki Reaction

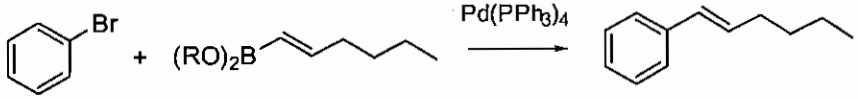


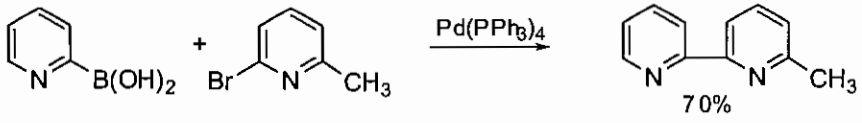






Example





Molecular Spectroscopy

electromagnetic radiation: energy that is transmitted through space in the form of waves

wavelength: (λ): the distance from the crest of one wave to the crest of the next wave

frequency: (ν): the number of complete cycles per second

$$\nu = \frac{c}{\lambda}$$ where c = speed of light

Electromagnetic radiation is transmitted in particle-like packets called photons or quanta. The energy is inversely proportional to the wavelength and directly proportional to frequency.

$$E = \frac{hc}{\lambda}$$ where c = speed of light; h = Planck' s constant

$$E = h\nu$$ h = Planck' s constant

ultraviolet visible infrared radio

decreasing energy

Absorbtion of ultraviolet light results in the promotion of an electron to a higher energy orbital.

Absorbtion of infrared results in increased amplitudes of vibration of bonded atoms.

Intensity of radiation is proportional to the number of photons.

MassSpectrometry

useful for determining molecular weight, presence of specific atoms and also certain molecular fragments

an organic molecule can be ionized by a number of methods such as bombardment by electron s or other high energy species.

usually the ionization results from loss of a single electron and the production of a cation radical.

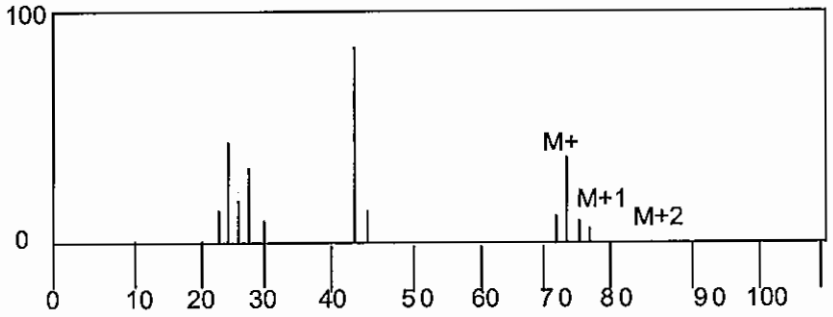
The princple of mass spectrometry is based on the fact that depending on the mass to charge ratio of a particular cation radical, it will travel along a different curved path when exposed to a magentic field.

$$\frac{m}{z} = \frac{H^2 r^2}{2V}$$

m = mass of cation radcal
z = charge (usually +1)
H = strength of the magnetic field
r = radius of the path
V = accelerating potential

placing a detector at some point along the flight path of the ion allows its mass to charge ratio to be calculated. Since almost all the ions will have a charge of +1, the mass to charge ratio is also the mass.

A mass spectrum produces a series of peaks which correspond to different mass of different molecular frgaments and their relative abundance



The **molecular ion** is the result of loss of one electron from the parent molecule. Sometimes the molecular ion is too unstable to be detected, but it usually is present in the mass spectrum.

The molecular ion also fragments into various other fragments by bond breaking processes in the gas phase.

Each of the fragments which reach the detector will produce a peak in the spectrum corresponding to its mass.

A peak in the region of highest m/ z in a mass spectrum often corresponds to the moecular ion.

I n addition to the peak for the molecular ion, there will also be peaks of M+1 and M+2 mass which correspond to similar ions which contain other isotopes of specific elements,

For example, the mass spectrum of 2-butanone contains a peak at 7 2 for the molecular ion $^{12}C_4{}^{1}H_8{}^{16}O$

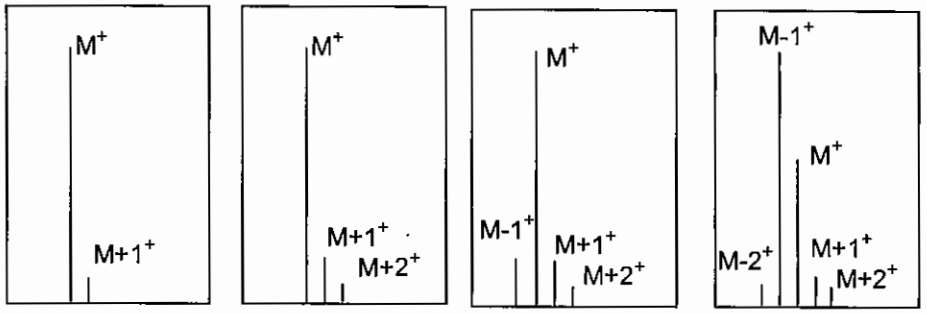
and a peak at 7 1 for other ions such as $^{13}C^{12}C_3{}^{1}H_8{}^{16}O$ or $^{12}C_4{}^{2}H^{1}H_7{}^{16}O$ or $^{12}C_4{}^{1}H_8{}^{17}O$

The **base peak** is the largest peak in the spectrum corresponding to the ion which is present in the greatest abundance.
The base peak is often the molecular ion, but not always.
The base peak can be the result of a fragmentation of the molecular ion into two other species.

To determine the molecular weight of a compound from the mass spectrum, first look at the region of hghest m/ z ratio.

I t is usually a reasonable assumption that one of these peaks will be the molecular ion.

I f the molecular ion is present and no Cl, BR or S are present in the molecule, one of four patterns are most common.



no $M+2^+$ present

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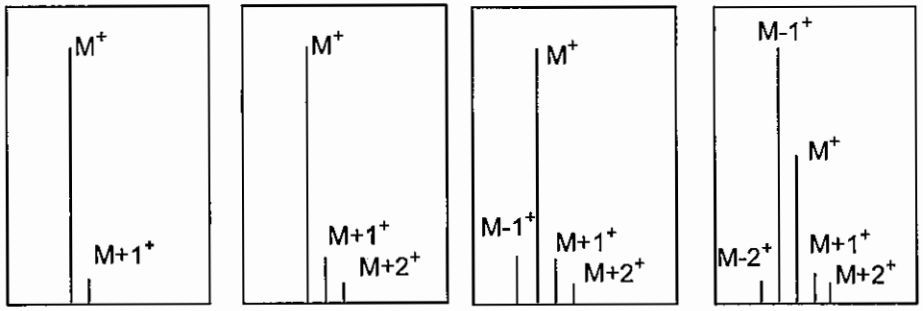


no $M+2^+$ present

I sotope	%
^{1}H	99.98
^{2}H	0.01
^{12}C	98.89
^{13}C	1.11
^{14}N	99.63
^{15}N	0.37
^{16}O	99.76
^{17}O	0.04
^{18}O	0.20
^{19}F	100.0

I sotope	%
^{31}P	100.0
^{32}S	95.00
^{33}S	0.76
^{34}S	4.22
^{36}S	0.01
^{35}Cl	75.53
^{37}Cl	24.47
^{79}Br	50.54
^{81}Br	49.46
^{127}I	100.0

Using the known relative abundance of isoptopes of different elements, the molecular formula can be deduced.

For example:

if the molecular ion is 68 , there are three reasonable possibilities

formula	M+1	M+2
$C_3H_4N_2$	4.07	0.06
C_4H_4O	4.43	0.28
C_5H_8	5.53	0.12

Assumes M+ is 100% otherwise it would be the specified pecentage of the M+ intensity.

Presence of Nitrogen

Determining the presence of nitrogen is very simple if there are an odd number of nitrogens present, because the molecular ion will be an odd mass.

For even numebrs of nitrogens, the M+, M+1, and M+2 intensties can be examined as illustrated before.

Presence of Sulfur

Determining the presence of sulfur can usually be determined the presence of a slightly large M+2 peak since ^{34}S is 4.22% abundant.

Presence of Bromin and Chlorine

Determining the presence of bromine and chlorine can also be determined from the M+2 peak since ^{37}Cl is 24.47 % abundant and ^{81}Br is 49 .46% abundant.

Thus the M+ and M+2 peaks in a compound containing chlorine will be about a 3:1 ratio and for one containing bromine M+ to M+2 will be about 1:1.

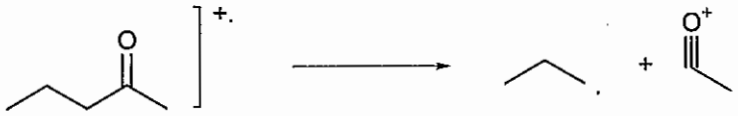
Fragmentation Patterns

cleavage at branches

because of cation stability, cleavage to produce stable cations is common

Fragmentation Patterns

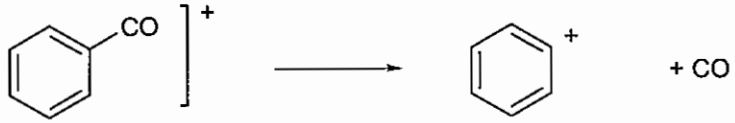
α,β-cleavage



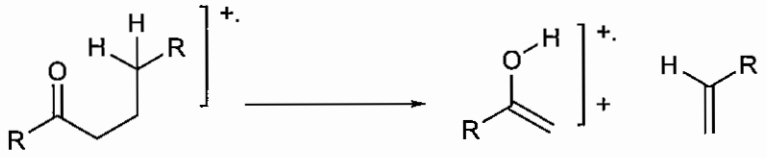
cleavage of a bond alpha and beta to a heteroatom such as oxygen is common in carbonyl compounds

Loss of a neutral molecule

Loss of H_2O, CO, HCN, HCl, NO, etc is common due to the stability of the neutral species



McLafferty Rearrangement



McClafferty rearrangment is very common in carbonyl compounds with a gamma hydrogen atom.

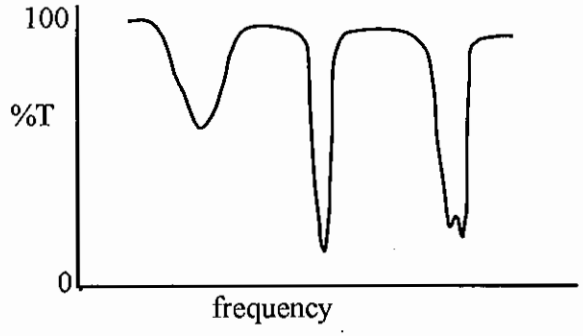
Infrared Spectroscopy

I nfrared is recorded as %T versus wavelength or freq uency

When a sample absorbs at a particular wavelength or freq uency, %T is reduced and a peak or band is displayed in the spectrum.

I nfrared is recorded as %T versus wavelength or freq uency

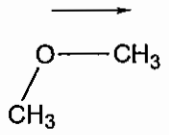
When a sample absorbs at a particular wavelength or freq uency, %T is reduced and a peak or band is displayed in the spectrum.



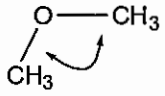
Nuclei of bonded atoms undergo vibrations similar to two balls connected by a spring. Depending on the particular atoms bonded to each other (and their masses) the freq uency of this vibration will vary.

I nfrared energy is absorbed by molecules resulting in an excited vibrational state. Vibrations occur in q uantized energy levels and thus a particular type of bond will absorb only at certain freq uencies.

Both stretching and bending vibrations can be observed by infrared.



stretching



bending

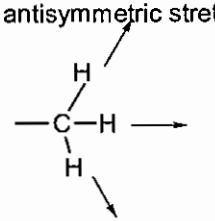
Frequency of vibration will be inversly proportional to the masses of the atoms.

C=S	C=O	C—H	C—D
1350 cm^{-1}	1700 cm^{-1}	3000 cm^{-1}	2200 cm^{-1}

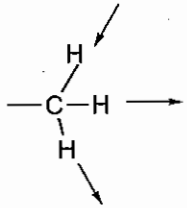
Frequency of vibration will be directly proportional to the strength of the bonds

C≡C	C=C	C—C
2150 cm^{-1}	1650 cm^{-1}	1200 cm^{-1}

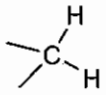
Some vibrations are coupled when atoms of similar masses are involved such as two or mopre C–H bonds such as in a methyl group where there are symmetric and antisymmetric stretches

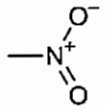


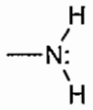
$C–H_{sym}$ = 2872 cm^{-1}



$C–H_{asym}$ = 2962 cm^{-1}







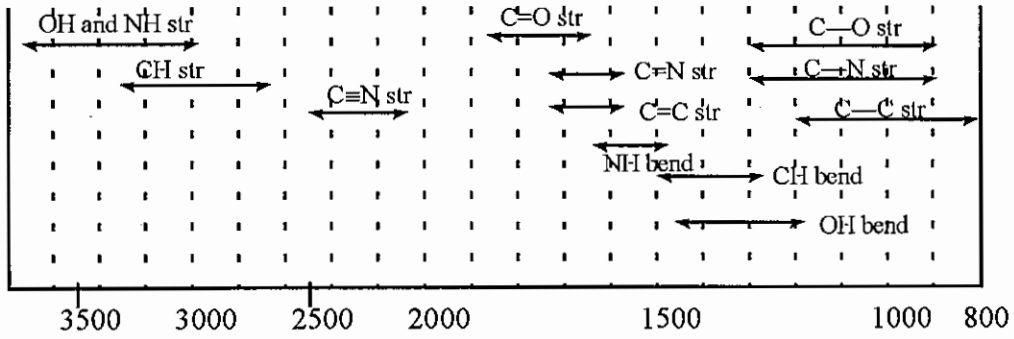
coupled vibrations are common as in functional groups above which each have a symmetric and antisymmetric vibration. These can help dentify certain functional groups

4000 cm^{-1} to 1300 cm^{-1} is known as the functional group region.
400 cm^{-1} to 1300 cm^{-1} is known as the fingerprint region since it is uniq ue for every compound.

Interpretation of Infrared Spectra

Correlation tables

I nfrared spectra of thousands of compounds have been tabulated and general trends are known. Some common functional groups are shown below.



Carbonyls

One of the most useful absorbtions in infrared 1640-18 20 cm-1

Ketones (saturated) C= O	1640-18 20 cm^{-1}
Aldehydes C= O;	1640-18 20 cm^{-1}
C—H(O)	28 20-29 00 and 27 00-27 8 0 cm^{-1} (weak but characteristic)
Carboxylic acids C= O;	1640-18 20 cm^{-1}
C(O) —OH	3330-29 00 cm^{-1}
Esters C= O ;	1640-18 20 cm^{-1}
C(O) —OR	1100-1300 cm^{-1}

C—C and C—H Bonds

sp^3 C—C	weak, not useful
sp^2 C= C	1600-17 00 cm^{-1}
sp^2 C—C (aryl)	145 0-1600 cm^{-1}
sp C≡C	2100-225 0 cm^{-1}
sp^3 C—H	28 00-3000 cm^{-1}
sp^2 C—H	3000-3300 cm^{-1}
sp C—H	3300 cm^{-1}
C(CH_3)$_2$	1360-138 5 cm^{-1} (two peaks)

Alcohols and Amines

O—H or N—H	3000-37 00 cm^{-1}
C—O or C—N	9 00-1300 cm^{-1}

Ethers

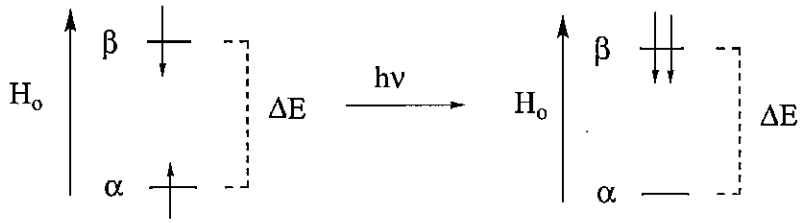
C—O	105 0-1260 strong

Nuclear Magnetic Resonance (NMR) Spectroscopy

Some atomic nuclei (^{1}H, ^{13}C, others) behave as if they are spinning...they have a nuclear spin.

Spinning of a charged particle creates a magnetic moment.

If an external magnetic field is applied, these small magnetic moments (of the nuclei) either align with the field (α) or against the field β) , about 50% with and 50% against the field at any one time.



H_o = the external magnetic field

Resonance: the flip of the magnetic moment from parallel to antiparallel to the external magnetic field.

Irradiation at the frequency equal to the energy difference ΔE, causes resonance.

ΔE depends on the external magnetic field.

Protons (or other nuclei) in different magnetic environments resonate at different field strengths.

A proton which resonates at a higher field is in a stronger magnetic environment or **shielded.**

A proton which resonates at a lower magnetic field is said to be **deshielded.**

Different magnetic environments are created by different electron densities in the vicinity of a proton.

Adj acent electron withdrawing groups, highly electronegative atoms, or the hybridization of the carbon to which the proton is bonded can alter the magnetic environment.

The local electrons create a small electric and magnetic field around a proton and shield it.

The more electron density present around the proton, the greater the field and the greater the shielding.

Resonances are reported in chemical shifts (δ) downfield from tetramethylsilane (TMS) ($C_4H_{12}Si$).

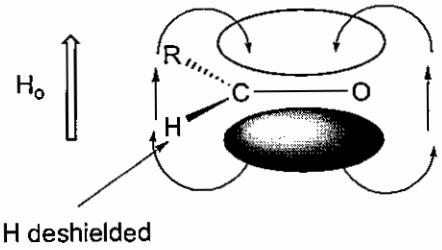
$$\delta = \frac{\text{distance from TMS in Hz}}{\text{MHz of spectrum}} \quad \text{ppm}$$

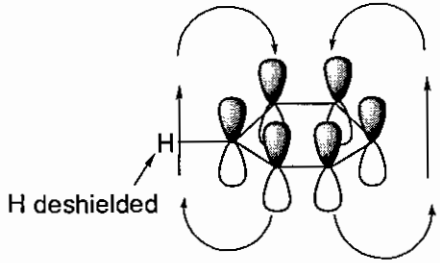
I n methyl halides, the more electronegative the halogen, the more deshielded the protons on the methyl. This is because F is inductively more electron withdrawing, causing the carbon to be more positive and thus pulling more electrons away from the hydrogen and causing it to be less shielded.

	H_3C—**F**	H_3C—**Cl**	H_3C—**Br**	H_3C—**I**
δ	4.3	3.0	2.7	2.1

Pi electron effects

Magnetic fields created by pi electrons are directional and said to have an anisotropic effect.





The pi system of benzene creates a magnetic field or ring current which deshields the protons attached to the ring.

Similarly, pi electrons in a C=O bond create a field which deshields the proton bonded to the C=O of an aldehyde. This is also affected by the inductive effect of the C=O.

I h methyl halides, the more electronegative the halogen, the more deshielded the p

Equivalent and Nonequivalent Protons

Protons that are in the same magnetic environment are equivalent and have the same chemical shifts.

Protons in different magnetic fields are nonequivalent and have different chemical shifts.

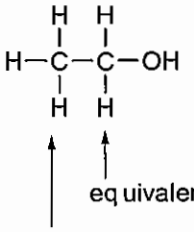
Magnetic equivalence is usually the same as chemical equivalence.

Equivalence can be established by symmetry operations such as rotation, mirror planes and centers of symmetry

Chemically equivalent protons have the same chemical shifts.

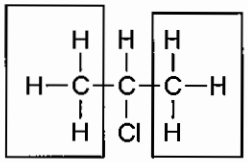
To determine if protons are chemically equivalent, replace one by a different group, e.g. D or Br.

Then replace a different one by the same group and compare the two compounds. If they are identical, the protons are equivalent.



equivalent, but not to CH_3 protons

equivalent



all six are equivalent

Equivalent protons can be on different carbons.

Protons which are **homotopic** or **enantiotopic** resonate at the same chemical shift in the NMR.

If protons are interconverted by rotation about a single bond, they will average out on the NMR time scale and a single resonance will be observed.

ClH_2CCH_2Cl anti and gauche forms rapidly interconvert and a single resonance is observed.

Axial and equatorial hydrogens in cyclohexane average to a single peak because of rapid ring inversion.

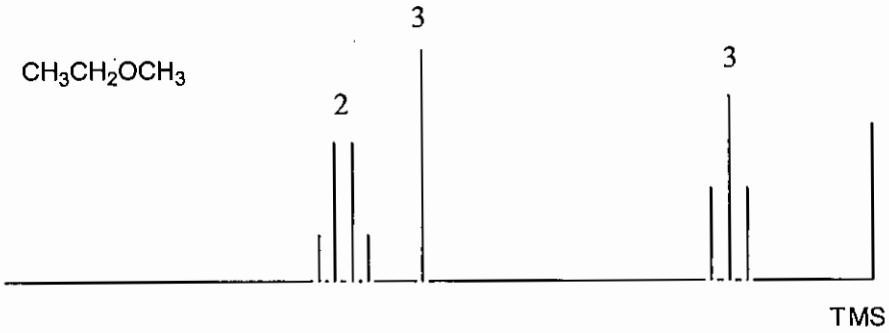
Diastereotopic hydrogens are chemically nonequivalent and thus give different chemical shifts in the NMR

Intergration

The spectrometer can integrate and determine the relative number of hydrogens associated with each resonance in the NMR spectrum by determining the area under the peaks.

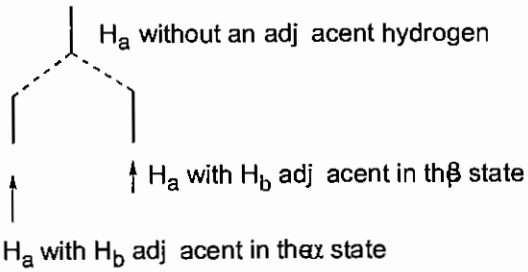
Spin-Spin Coupling

for example...



I f a proton (H_a) is bonded to a carbon which is bonded to a carbon that has one proton (H_b) , Ha will appear as a doublet

Since in half the molecules, H_b will be in the α state and in half will be in the β state, H_a will experience two different magnetic fields and two peaks (**a doublet**) will appear for H_a.



For one adjacent hydrogen
α or β

For two adjacent hydrogens: Hb, Hc

At any one time Hb or Hc could be in the α or β state (50:50) thus 4 combinations for Hb, Hc exist:

$\alpha_b\alpha_c$ $\alpha_b\beta_c$ $\beta_b\beta_c$ gives 1:2:1 triplet
$\beta_b\alpha_c$

When both Hb and Hc are α, a different field is observed than if both are β or one is α and one is β.

When one is α and one is β, the field is the same. That is, $\beta_b\alpha_c$ and $\alpha_b\beta_c$ produce the same field and a single signal for Ha is observed with twice the intensity.

Thus three signals are observed in a 1:2:1 ratio: a so-called triplet

For three adjacent protons:

$\alpha\alpha\alpha$ $\alpha\alpha\beta$ $\alpha\beta\beta$ $\beta\beta\beta$ 1:3:3:1 quartet
$\alpha\beta\alpha$ $\beta\alpha\beta$
$\beta\alpha\alpha$ $\beta\beta\alpha$

Thus the splitting pattern of a particular proton or equivalent protons will be a pattern with n+1 lines where n is the number of adjacent equivalent protons.

singlet 0 neighboring protons
doublet 1 neighboring protons
triplet 2 neighboring protons
quartet 3 neighboring protons
quintet 4 neighboring protons
sextet 5 neighboring protons
septet 6 neighboring protons

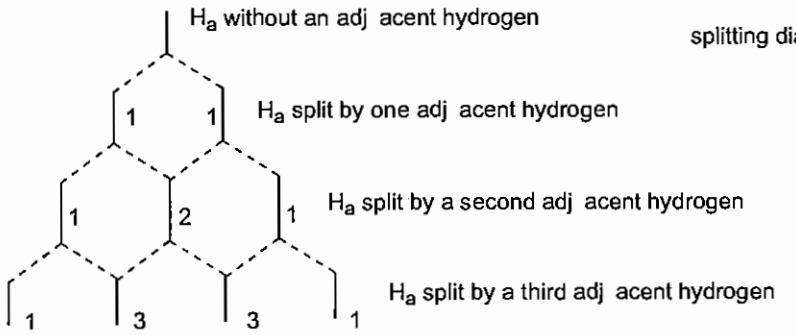
The separation of the peaks in a splitting pattern is called the **coupling constant, J.**

Splitting Diagrams

Splitting patterns for protons can be constructed in diagram form by starting with one line to represent the unsplit proton resonance.

I f an adj acent proton Hb affects Ha it is split into a doublet; if another eq uivalent proton to Hb is present, each line of the double will be split into a doublet, since the coupling constant J is the same, the two center lines overlap and a only three lines are observed with the center line twice the height.

This can be repeated for additional adj acent protons.



Chemical Exchange and Hydrogen Bonding

CH_3OH, methanol would be expected to give an NMR spectrum of a doublet for the CH_3 and a q uartet for the OH. For a dilute sample at -40° in CCl4 this is the case.

I f the NMR spectrum is run at 25° as a more concentrated sample only two singlets are observed. This is because the intermolecular hydrogen bonding in methanol allows the rapid exchange of the OH proton from one CH_3OH molecule to another, effectively averaging the spin states of the OH proton and resulting in no change in the magnetic field due to the OH.

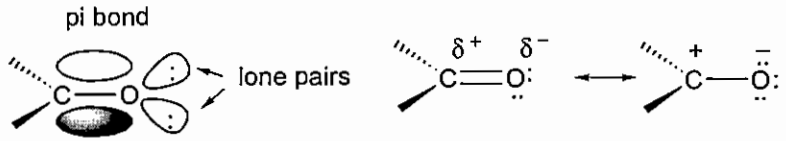
Amines and other compounds which can undergo hydrogen bonding can also show this effect. Thus the NMR spectra of alcohols, amines and carboxylic acids are temperature, concentration and solvent dependent.

CHEMICAL SHIFTS

Functional Group	Shift, δ
Primary alkyl, RCH_3	0.8-1.0
Secondary alkyl, RCH_2R	1.2-1.4
Tertiary alkyl, R_3CH	1.4-1.7
Allylic, $R_2C{=}C{-}CH_2R$	1.6-1.9
Benzylic, $ArCH_2R$	2.2-2.5
Iodoalkane, RCH_2I	3.1-3.3
Bromoalkane, RCH_2Br	3.4-3.6
Chloroalkane, RCH_2Cl	3.6-3.8
Ether, RCH_2OR	3.3-3.9
Alcohol, RCH_2OH	3.3-4.0
Ketone, $RCH_2C(=O)R$	2.1-2.6
Aldehyde, $RCH(=O)$	9.5-9.6
Terminal alkene, $R_2C{=}CH_2$	4.6-5.0
Internal alkene, $R_2C{=}CHR$	5.2-5.7
Aromatic, $Ar{-}H$	6.0-9.5
Alkyne, $RC{\equiv}C{-}H$	1.7-3.1
Alcoholic hydroxy, ROH	0.5-5.0 (variable)
Amine, RNH_2	0.5-5.0 (variable)

Carbonyl Group

sp3 hybridized, trigonal planar carbonyl carbon; partial positive C and partial negative (Lewis basic) oxygen.



C–O pi* is low-lying and therefore interacts well with high-lying filled-nonbonding orbitals: thus nucleophilic, not electrophilic addition reactions are charactersistic of carbonyl compounds.

Because of the polar C= O bond, boiling points are higher than nonpolar compounds of similar molecular weights.

Aldehydes and ketones are capable of hydrogen bonding to water, alcohols and acids

Spectral Properties

I nfrared:
Ketones(C=O) 1660-1750 cm-1
shifted by 25 cm-1 if aromatic or unsaturated :
PhCHO, CH_2=CHCOCH$_3$

Adlehydes (C=O) 1700-1740 (C—H) 2850

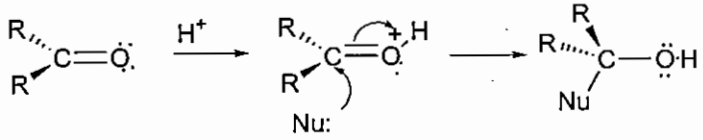
^{1}H NMR R—CHO 9-10 ppm
RCH_2COR 2.0 - 2.6 ppm due to inductive deshielding

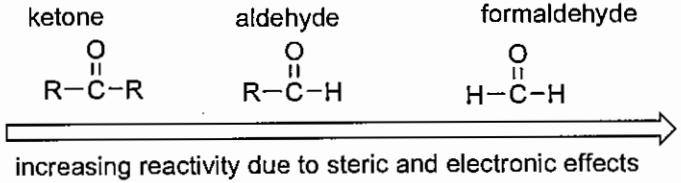
Nucleophilic addition reactions

Addition Reactions of Aldehydes and Ketones

Carbonyl group can be attacked by nucleophiles

or undergo addition of reagents to the pi bond by electrophiles adding first





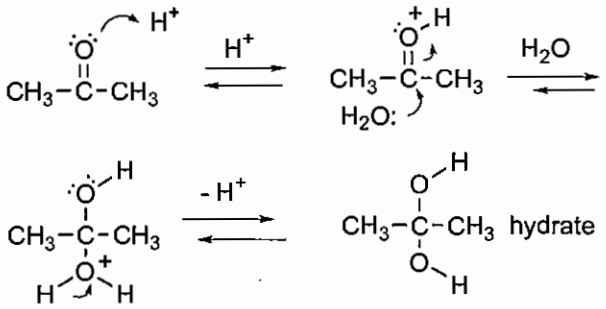
Ketones are more sterically hindered since they have two alkyl groups; aldehydes have one H and one alkyl group.

Alkyl groups are electron releasing and make the C=O carbon less positive.

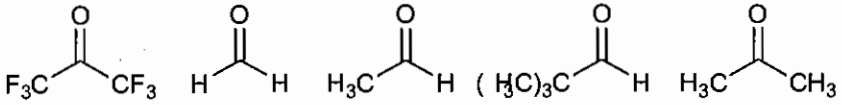
Reaction with H_2O: Formation of Hydrates

hydrates are normally transient, unstable species which are in equilibrium with the carbonyl compound

Acid catalyzed mechanism



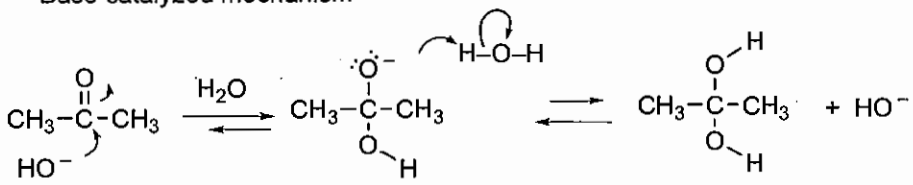
the eq uilibrium constant for the formation of hydrates is dependent on the carbonyl substituents: the more sterically hindered and the more electron rich the carbonyl, the less of the hydrate that will be present, conversely, the less sterically hindered and the more electron deficient the carbonyl carbon, the more hydrate that will be present.



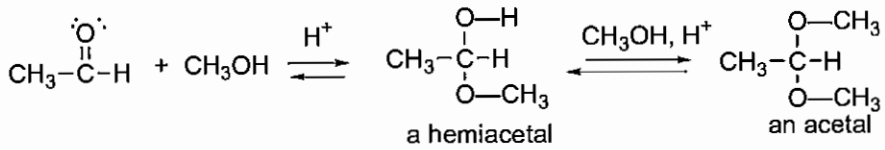
22,000	41	1.8×10^{-2}	4.1×10^{-3}	2.5×10^{-5}

$K_{hydration}$

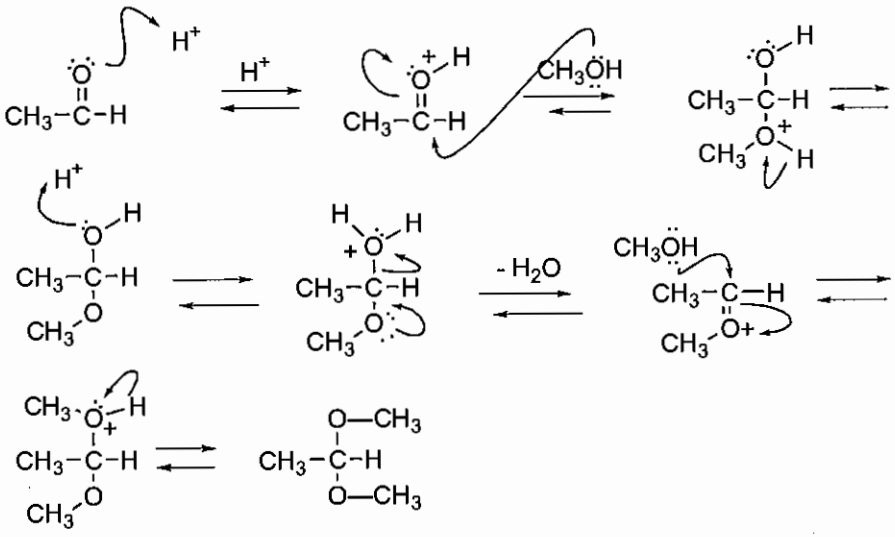
Base catalyzed mechanism



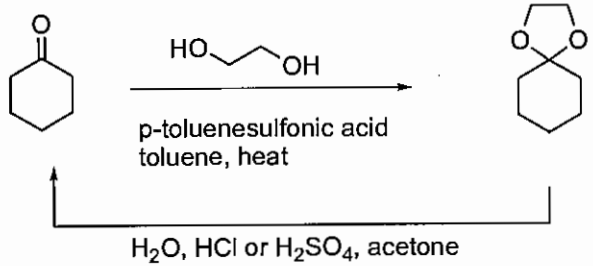
with alcohols: formation of acetals



mechanism

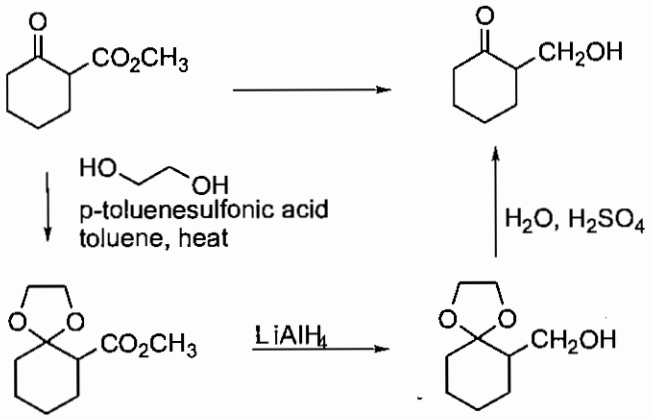


if H_2O is present in a large amount, the carbonyl compound will be favored.
if H_2O is not present, but ROH is present, the acetal will be favored

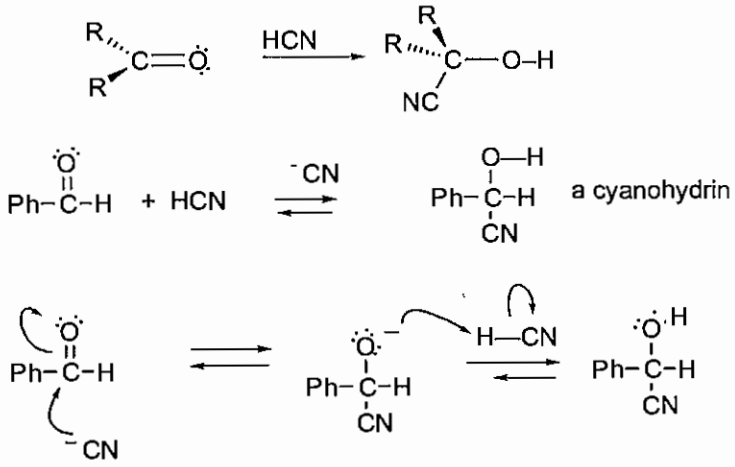


Acetals as protecting groups

many times multifunctional compounds must be treated to convert one functional group selectively. In the example below, direct treatment of the keto-ester with $LiAlH_4$ would result in reduction of both carbonyls, so the ketone must be protected prior to reduction of the ester



Formation of cyanohydrins

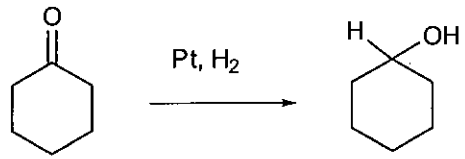


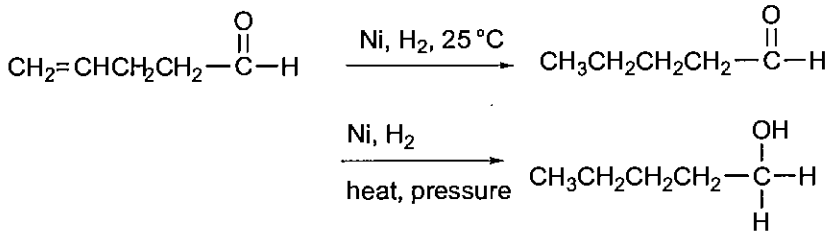
forms a new C–C bond and introduces a functional group which can be converted to a carboxylic acid or an amine

Reduction of Carbonyl Compounds

a) Hydrogenation

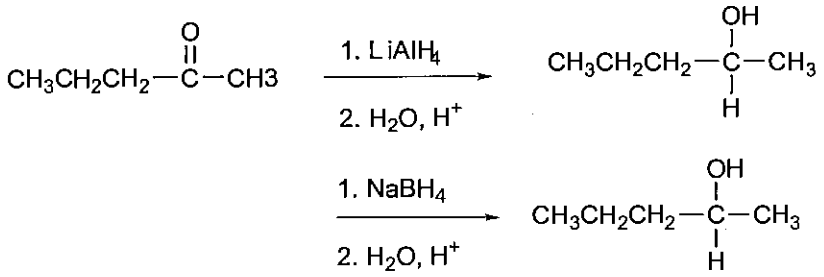
C=O bond can be hydrogentated much like a C=C bond, C=O usually requires harsher conditions





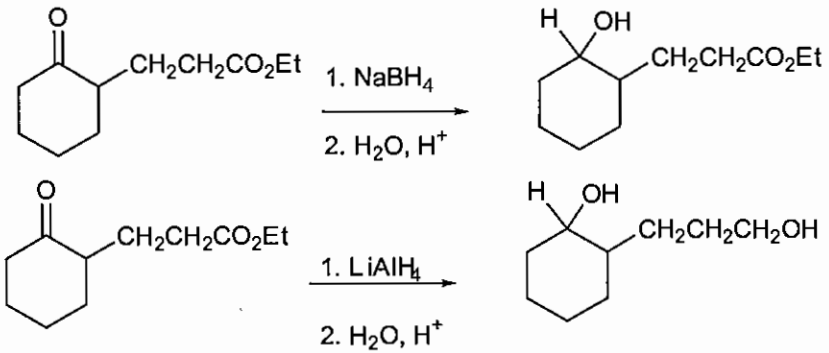
b) Metal Hydrides

$LiAlH_4$, lithium aluminum hydride
$NaBH_4$, sodium borohydride



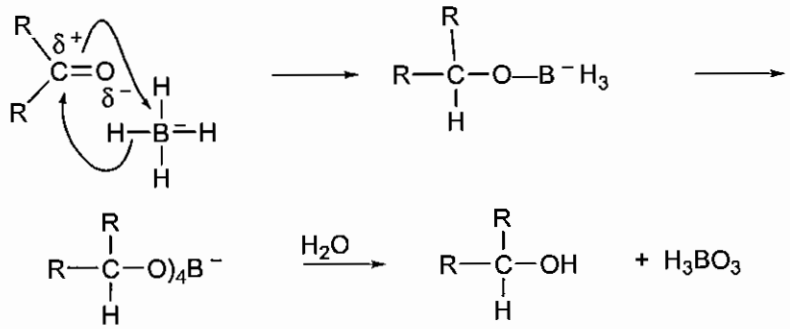
$LiAlH_4$ much more reactive also reduces esters, carboxylic acids, nitriles, amides
$NaBH_4$ sodium borohydride reduces only aldehydes and ketones: more selective

For example

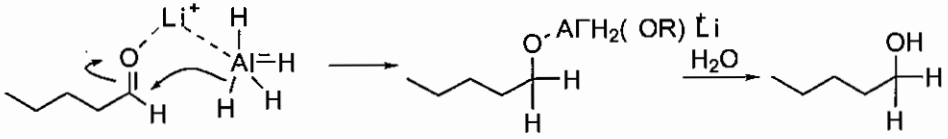


$LiAlH_4$ and $NaBH_4$ do not reduce isolated double bonds

Mechanism for $NaBH_4$:

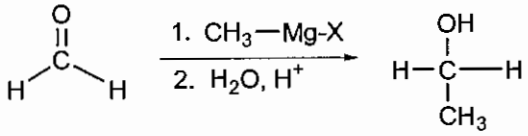


Mechanism for $LiAlH_4$:

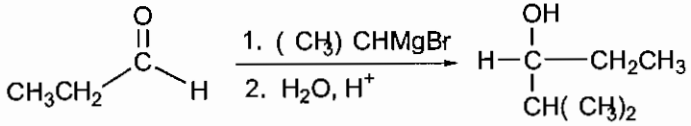


A Grignard reaction with

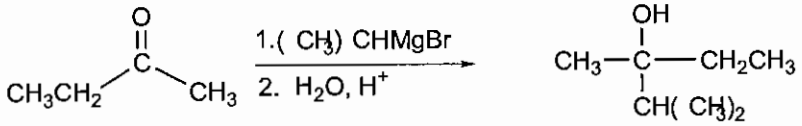
1. formaldehyde produces a primary alcohol



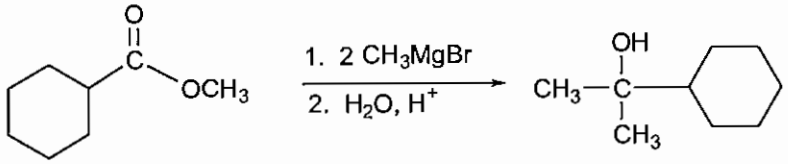
2. an aldehyde produces a secondary alcohol



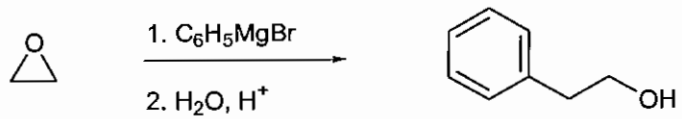
3. a ketone produces a tertiary alcohol



4. an ester produces a tertiary alcohol (addition of two molecules of Grignard reagent)



5. ethylene oxide produces a primary alcohol



Carbohydrates are naturally occurring compounds with C, H, O; often with the emperical formula CH_2O.

Monosaccharides...simple sugars which cannot be broken down by hydrolysis; e.g. glucose, fructose, ribose, galactose, deoxyribose, etc.

Disaccharides...dimers of monosaccharides units; e.g.sucrose is made up of glucose and fructose

Oligosaccharides...two to eight monosaccharides units

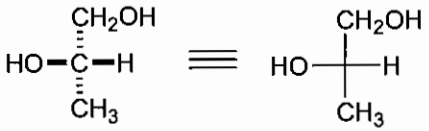
Polysaccharides...more than eight monosaccharide units; e.g. cellulose is polyglucose

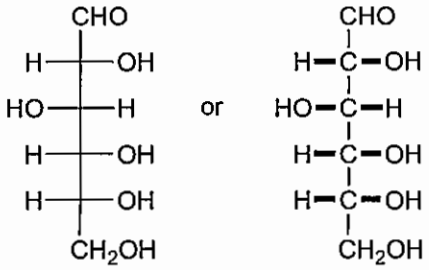
Fischer Projections

for simplicity carbohydrates are often represented by Fischer Proj ections.

in a Fischer proj ection the horizontal bonds are always out and the vertical bonds are always in.

Fischer Proj ections may be rotated 180° but not 90°.
A 90° rotation creates the enantiomer.



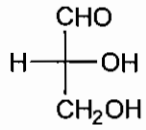


Classification of Carbohydrates

The ending-ose indicates a carbohydrate
An aldose contains and aldehyde; a ketose contains a ketone
A triose has three carbons, a tetrose has four carbons, a pentose has five carbons and a hexose has six carbons.
A ketohexose is a six carbon sugar containing a ketone.

D and L sugars

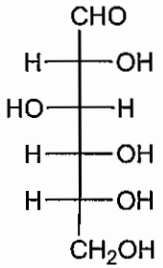
I n the 19th century (+) -glyceraldehyde was arbitrarily assigned the configuration below and designated D.



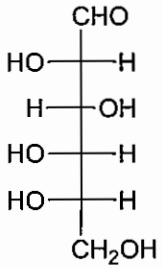
D-glyceraldehyde

Now all carbohydrates with a hydroxyl to the right on the last carbon in the Fischer

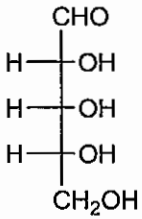
Proj ection is designated D. I f the OH is proj ected to the left, the sugar is an L sugar.



D-glucose



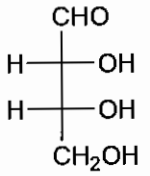
L-glucose



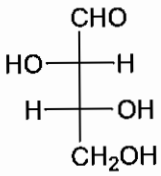
D-ribose

Aldohtetroses

The aldotetroses have 2 asymmetric carbons and thus 2^2 or 4 stereoisomers. The D- seres is shown below. Each has a corresponding L -isomer



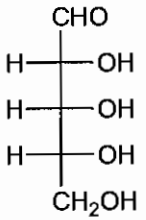
D-erythrose



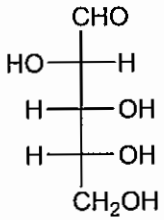
D-threose

Aldopentoses

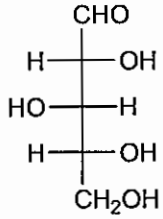
The aldotetroses have 3 asymmetric carbons and thus 2^3 or 8 stereoisomers. The D- seres is shown below. Each has a corresponding L -isomer



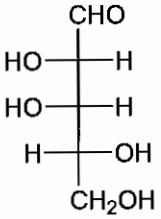
D-ribose



D-arabinose



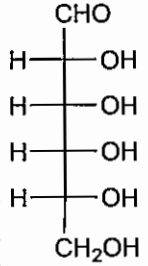
D-xylose



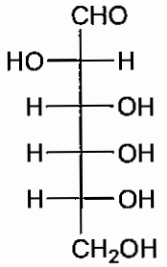
D-lyxose

Aldohexoses

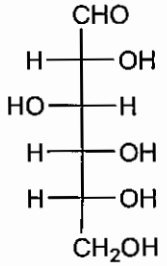
The aldohexoses have 4 asymmetric carbons and thus 2^4 or 16 stereoisomers. The D- seres is shown below. Each has a corresponding L -isomer



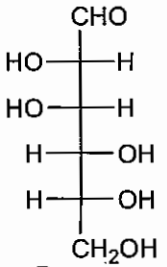
D-allose



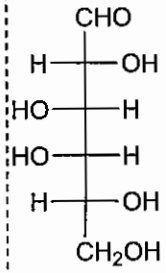
D-altrose



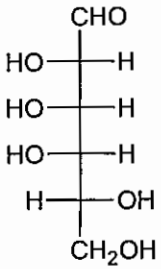
D-glucose



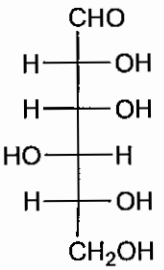
D-mannose



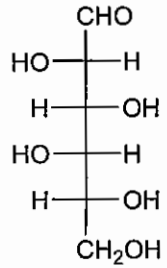
D-galactose



D-talose

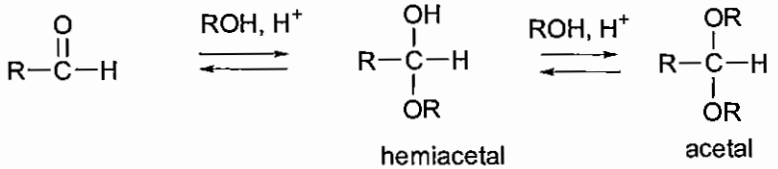


D-gulose

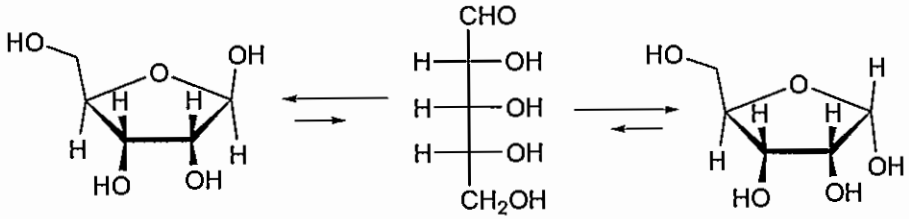


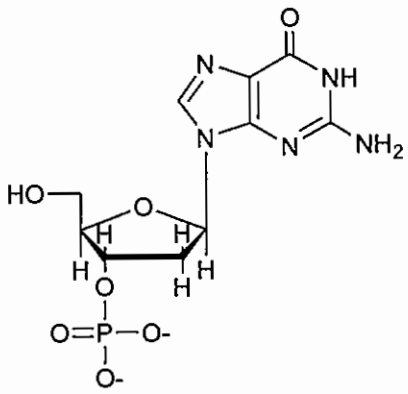
D-idose

The open chain representations of the sugars shown above is for simplicity. Sugars normally exist as cyclic hemiacetals.

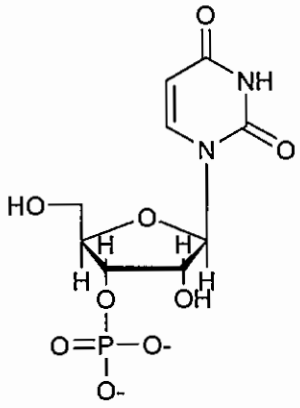


Furanose Forms



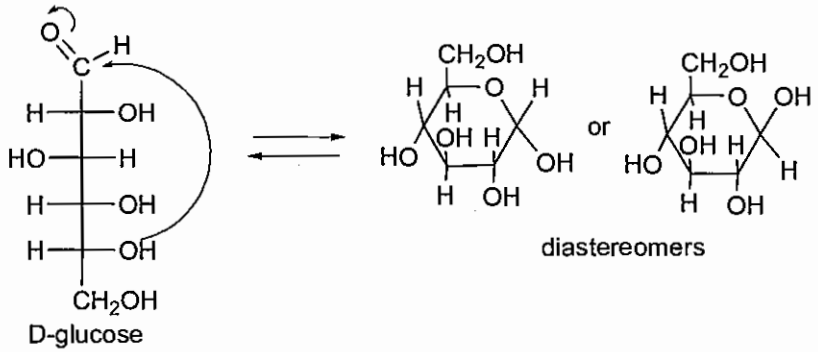


a DNA nucleotide

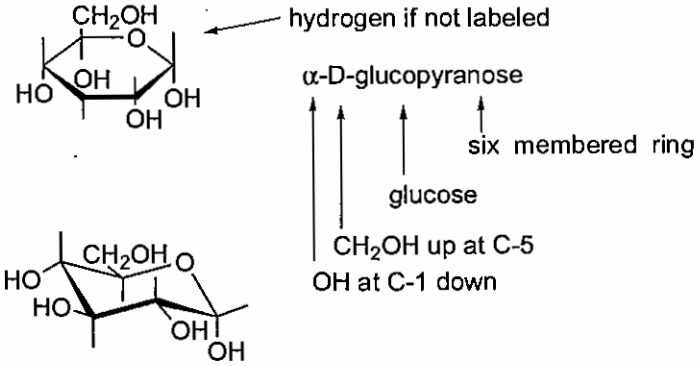


an RNA nucleotide

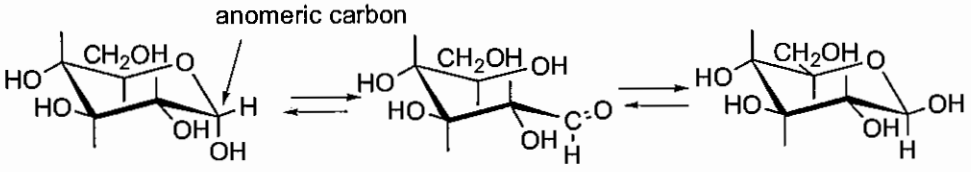
Pyranose Forms



Haworth projection



Anomers: monosaccharides which differ only in their configuration at C-1



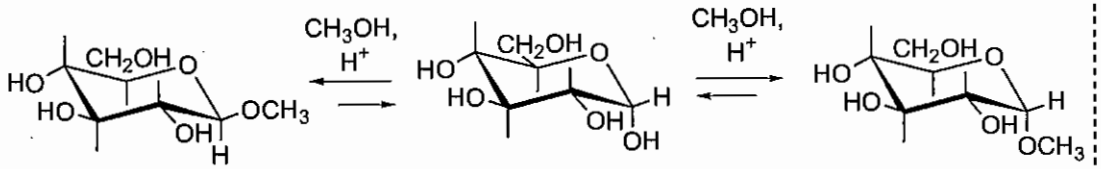
Mutarotation

α-D-glucose has a melting point of 146° and a specific rotation of +112°.
β-D-glucose has a melting point of 150° and a specific rotation of 18.7°.

The specific rotation of a solution of either α or β-D-glucose slowly changes until it reaches an equilibrium value of +52.6°.

This is mutarotation and is due to the conversion of α-D-glucose to β-D-glucose or the reverse.

The two forms are in equilibrium in solution: 36%α and 64% β.

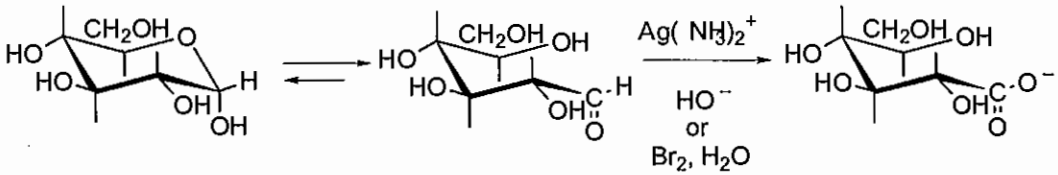


Glycosides are stable under neutral and basic conditions but are interconverted in acid.

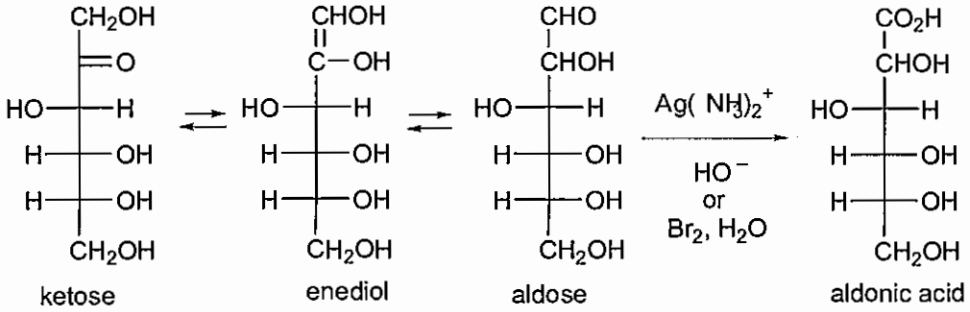
Oxidation

Aldoses are readily oxidized because the hemiacetal is in equilibrium with an aldehyde, a readily oxidized functional group.

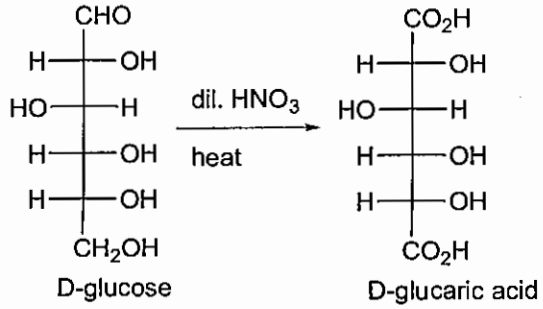
The product of the oxidation of the aldehyde of an aldose to a carboxylic acid is called an aldonic acid.



Even ketoses can be oxidized since they are in equilibrium with the aldose through an enediol tautomer



The product of oxidation of the aldehyde and the terminal primay alcohol to form a diacid is called an aldaric acid.

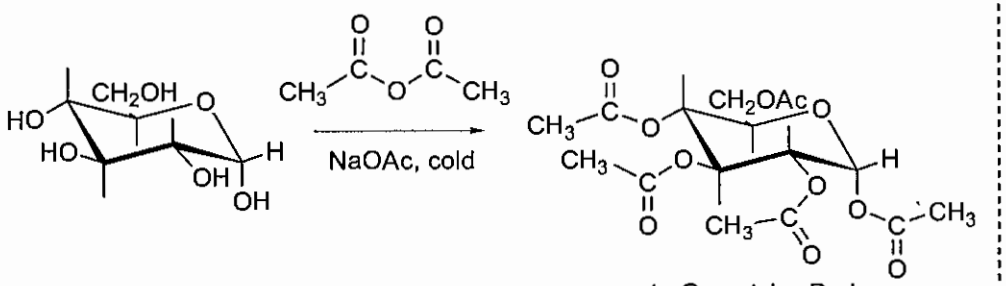


A uronic acid results when the terminal primary hydroxyl has been oxidized to a carboxylic acid. This generally only occurs enzymatically.

Reactions of the Hydroxyl Groups

The alcohols in sugars react much like any hydroxyl functional group.

Ester formation from acetic anhydride



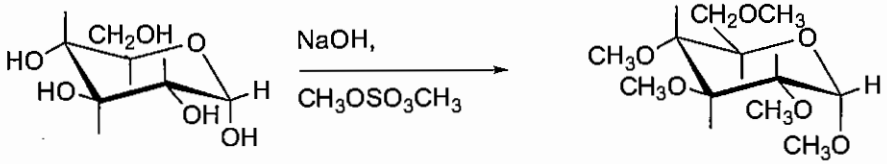
penta-O-acetyl-α-D-glucopyranose

Ether Formation

The hydroxyls of carbohydrates are more acidic than normal alcohols because of the inductive effects of the adj acent oxygens.

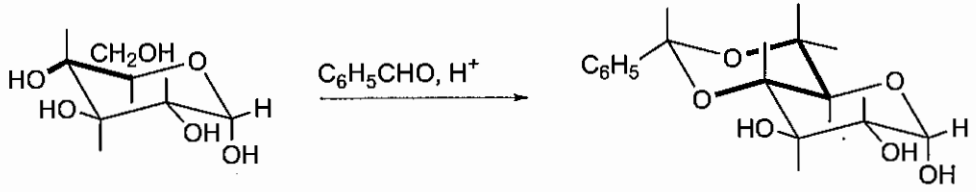
These hydroxyls can be deprotonated to form alkoxides with NaOH.

The alkoxides can be alkylated (S_N2 displacement) by dimethyl sulfate...sulfate is an excellent leaving group because of the resonance stabilization of the anion.



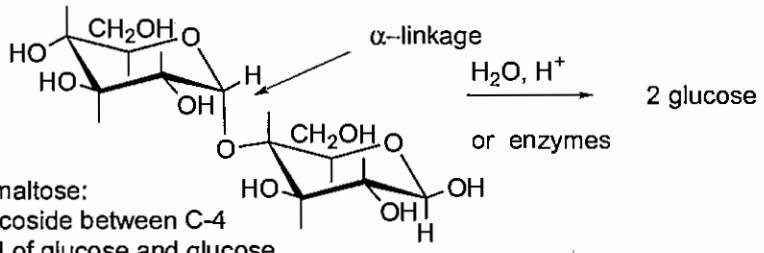
all hydroxyls are converted to methyl ethers

Acetal Formation



Disaccharides

Maltose

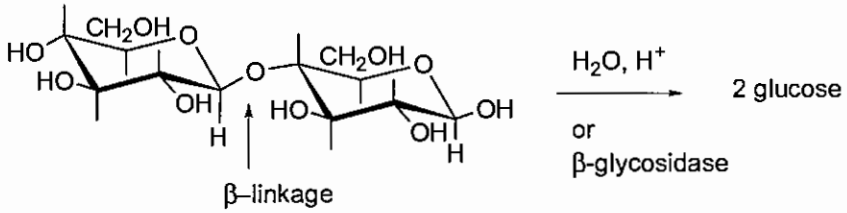


α-maltose:
glycoside between C-4 OH of glucose and glucose anomeric carbon

Starch $\xrightarrow[\text{maltohydrolase}]{\alpha\text{-1,4-glucan}}$ maltose $\xrightarrow{\alpha\text{-glucosidase}}$ glucose $\xrightarrow{\text{enzymes}}$ beer

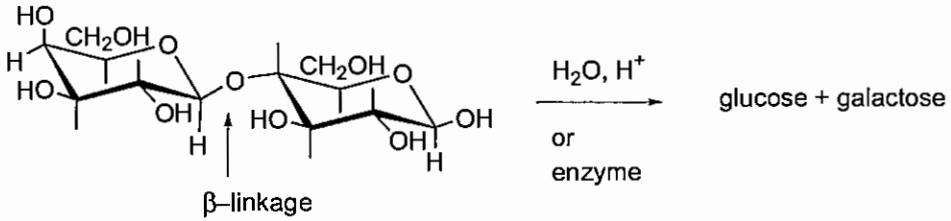
Cellobiose

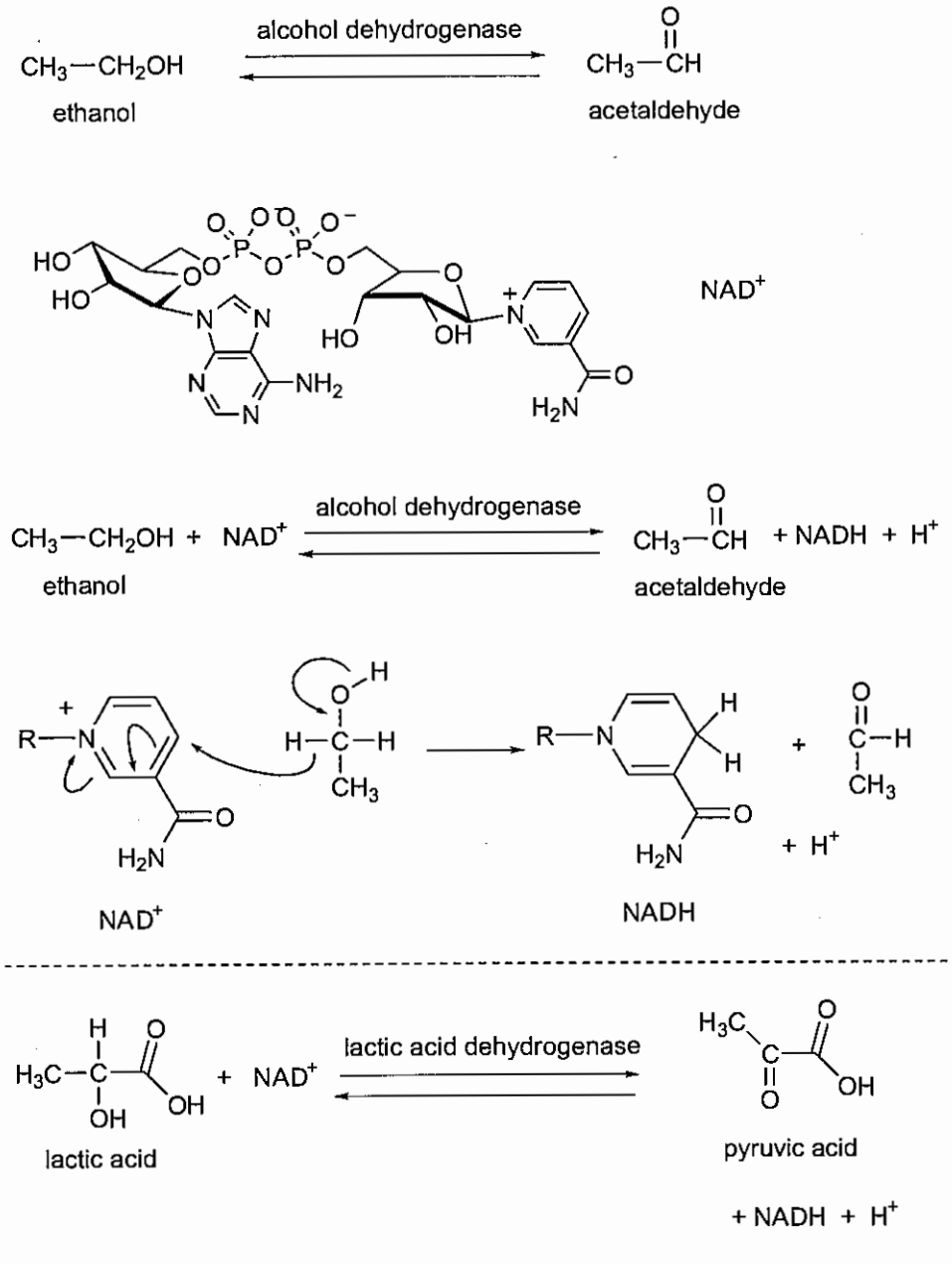
principal disaccharide of cellulose



Lactose

only in mammals, 5% in human milk; one unit of glucose, one of galactose

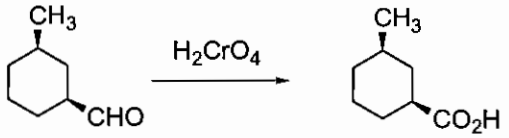




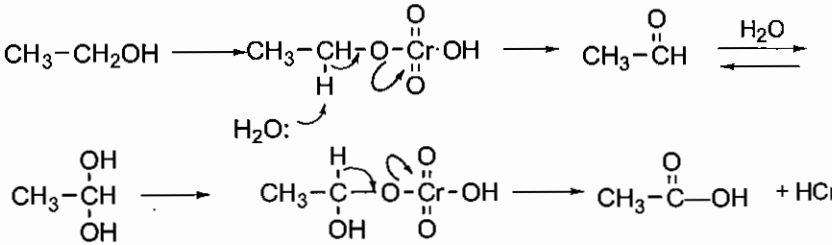
Oxidation of Aldehydes

Aldehydes can be oxidized to carboxylic acids by $KMnO_4$ or H_2CrO_4.
Ketones cannot ordinarily be oxidized further.

$CH_3CH_2CH_2CH_2CHO + KMnO_4, H^+, H_2O \longrightarrow CH_3CH_2CH_2CH_2CO_2H$

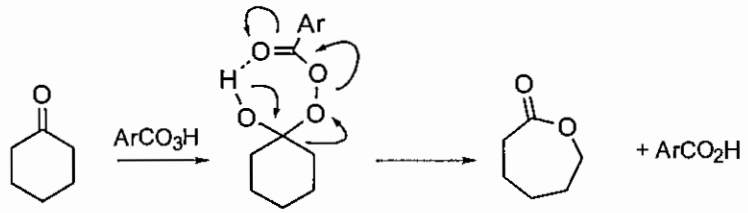


Aldehydes are oxidized to carboxylic acids throught their hydrates

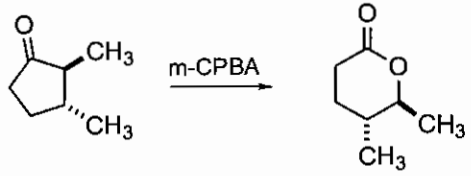


Baeyer-Villiger Oxidation

oxidation of a ketone to an ester (cyclic ketone to a lactone)

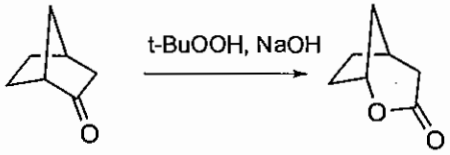


most substituted carbon (best able to stabilize a positive charge; i.e. one with highest electron density) will migrate.

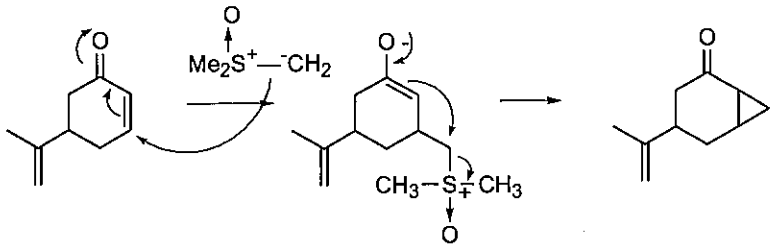


retention of configuration

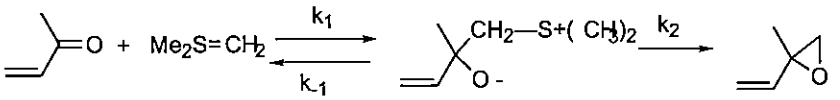
Strained systems can be oxidized with H_2O_2, HO^- or t-BuOOH, HO^- and do not require RCO_3H



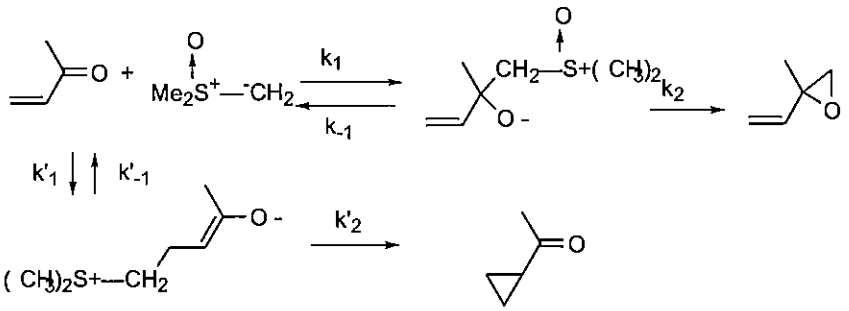
Unstabilized sulfur ylides reaction with unsaturated ketones to give epoxides:



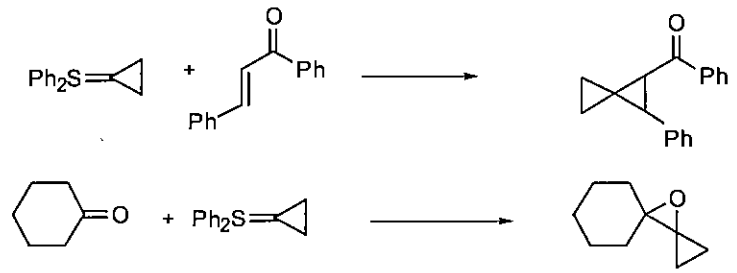
Explanation:



unstabilized sulfur ylides: k-1 < k2
in stabilized sulfur ylides k-1 > k2; k' 2 > k' -1



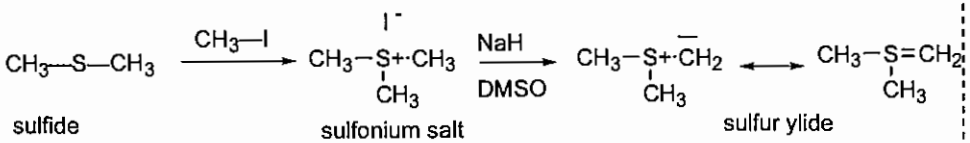
Cyclopropylidine sulfur ylides

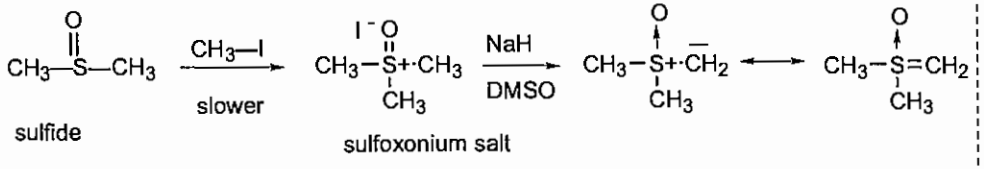


Trost J. Am. Chem. Soc.**1973**, 95, 5298, 5307, 5311, 5317.

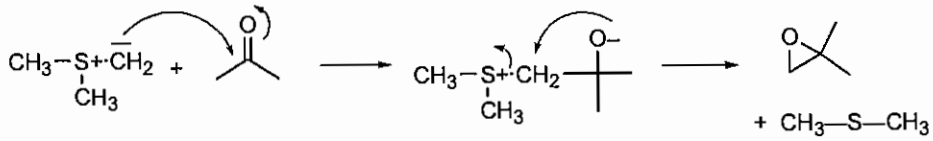
Sulfur Ylides

Sulfur ylides react with aldehydes and ketones to give epoxides rather than alkenes

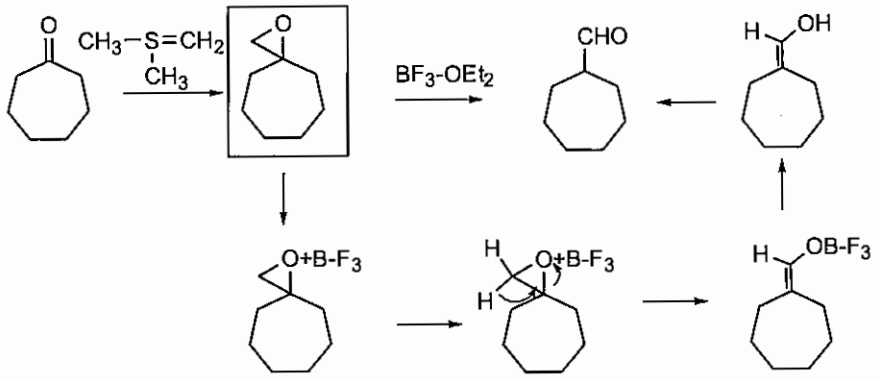




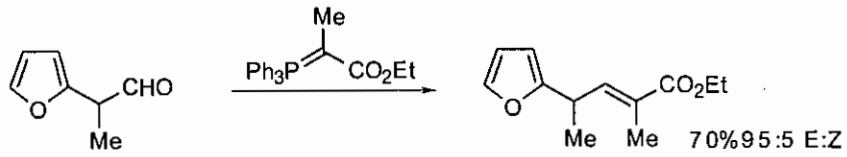
oxygen anion in intermediate displaces the sulfide to form an epoxide



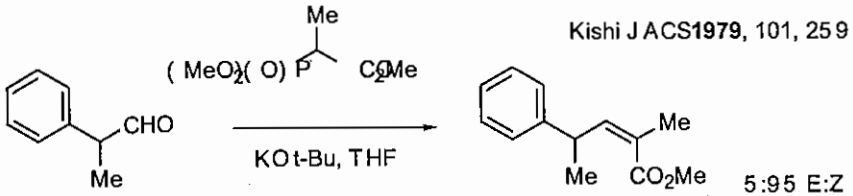
Examples:

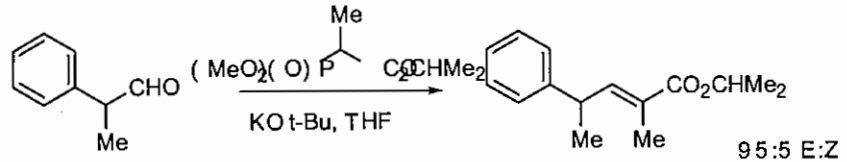


Stereocontrol in the Wadsworth-Emmons

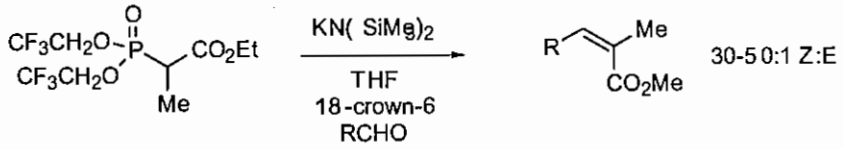


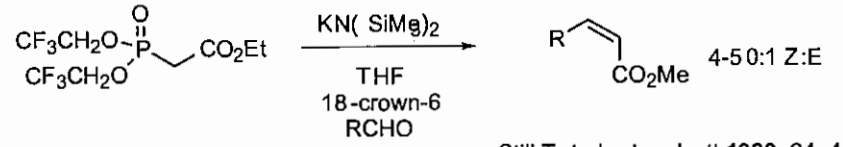
Kishi J ACS**1979**, 101, 259.



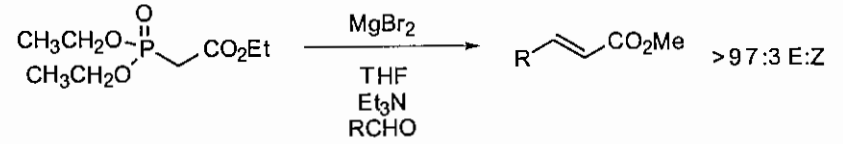


Kishi Tetrahedron Lett.**1981**, 37, 3873.





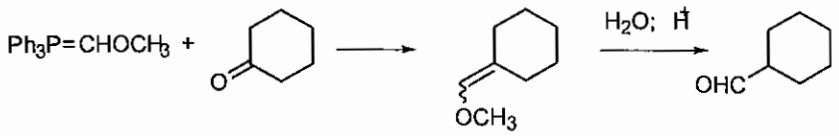
Still Tetrahedron Lett.**1983**, 24, 4405.



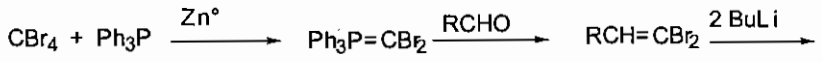
Rathke J. Org. Chem**1986**, 50, 2624.

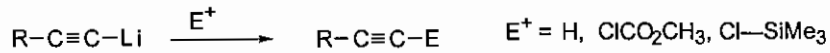
Special Ylides

one carbon homologation:



Corey-Fuchs



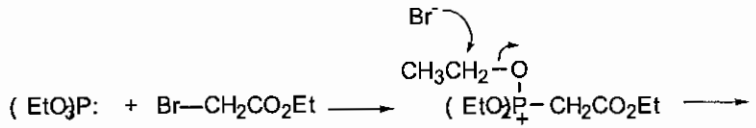


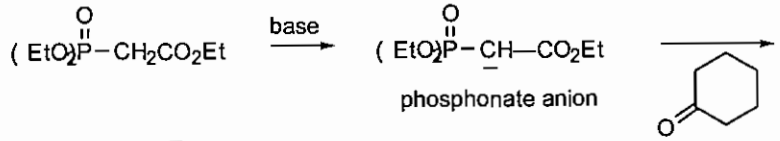
Wadsworth-Emmons Reaction

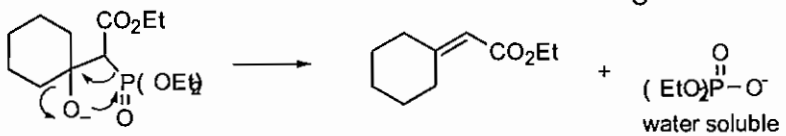
Uses phosphonate anions instead of ylides

Nucleophile is an anion, not an ylide and it is thus more reactive

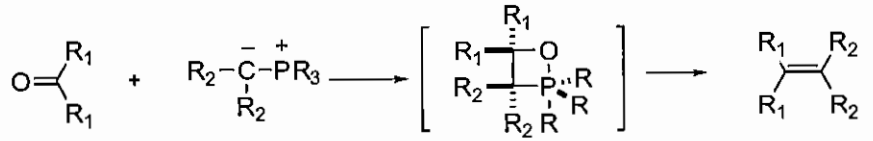
Phosphonate is formed by the reaciton of a trialkyl phosphite and and alkyl halide: the Arbuzov Reaction:







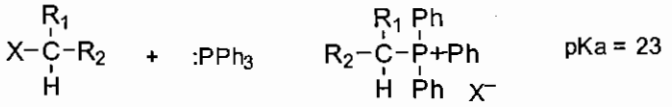
The Wttig Reaction



carbonyl phosphorous ylide + $Ph_3P=O$

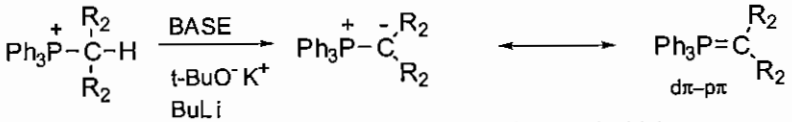
Driving force is the formation of the very strong P—O bond

Phosphonium salts are readily formed from triphenyl phosphine and primary or secondary alkyl halides. Tertiary alkyl halides are not useful since the reaction is an SN2 reaction.



pKa = 23

X = I , Br, OTs



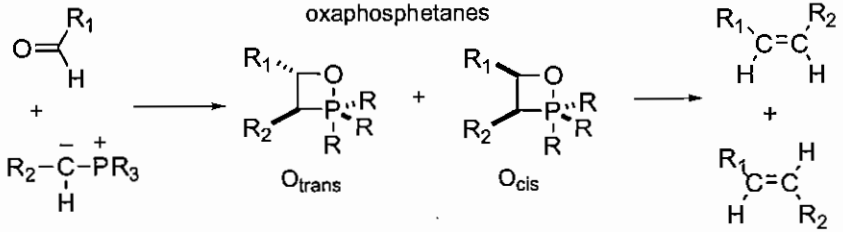
BASE

t-BuO⁻ K^+
BuLi
NaH
EtO^- Na^+
$CH_3SOCH_2^-$

Phosphorane (ylide)

Acidity of carbon adjacent to $^+PPh_3$ is due to a combination of inductive and resonance effects. C lone pair P-antibonding overlap.

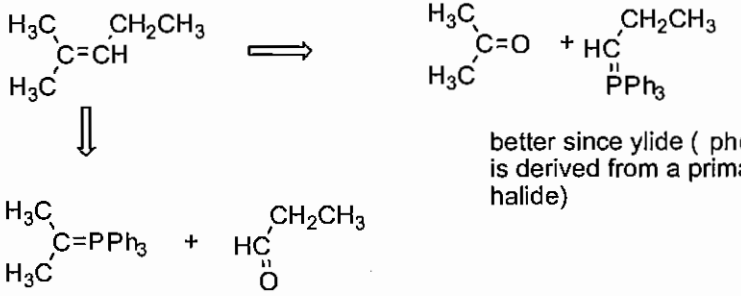
Mechanism:



oxaphosphetanes

O_{trans} O_{cis}

The position of the alkene is unambiguous in the product.

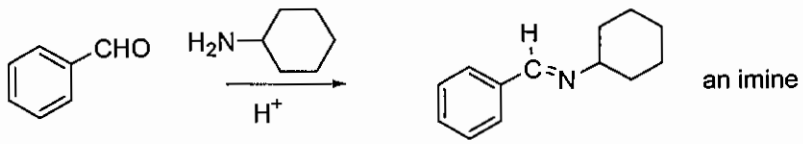


better since ylide (phosphorane is derived from a primary halide)

ylide derived from a secondary halide: substitution will be more difficult.

Addition-Elimination Reactions

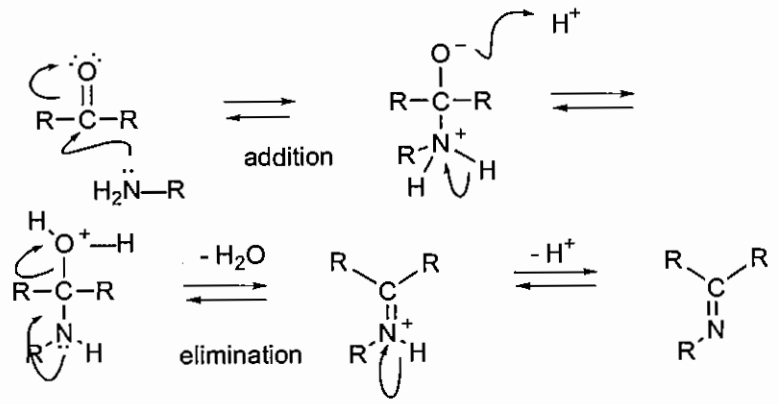
primary amines produce I mines



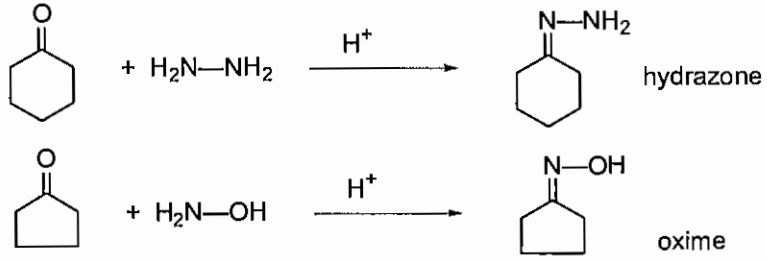
an imine

if the reaction is too acidic the amine is completely protonated and will not add
if the reaction is not acidic, OH_2 is not eliminated

mechanism of imine formation

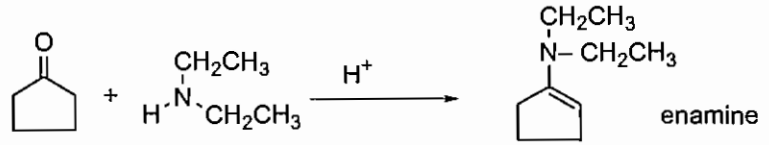


Hydrazones and Oximes



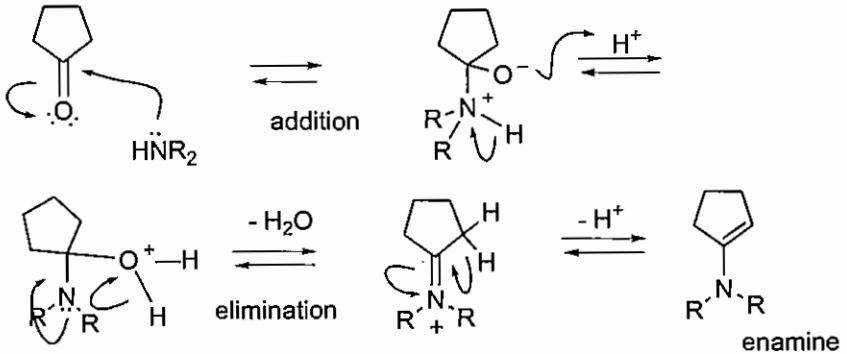
oximes, phenyl hydrazones, etc. are often solid and can be used to characterize carbonyl compounds by melting points

secondary amines produce enamines



enamine

mechanism of enamine formation



enamine

Nitriles

CH_3CO_2H acetic acid
$CH_3CH_2CH_2CH_2CO_2H$ pentanoic acid

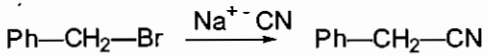
CH_3CN acetonitrile
$CH_3CH_2CH_2CH_2CN$ pentanonitrile

—C≡N: $pK_b = 24$ NH_3 $pK_b = 4.5$

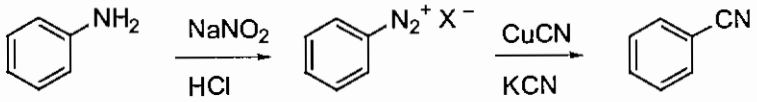
electrons more tightly held in sp orbital, 50% s character N more electronegative

Preparation

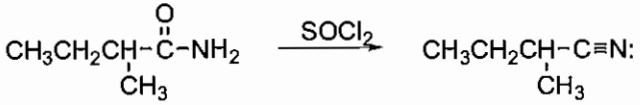
SN_2 with cyanide ion



From benzenediazonium salts

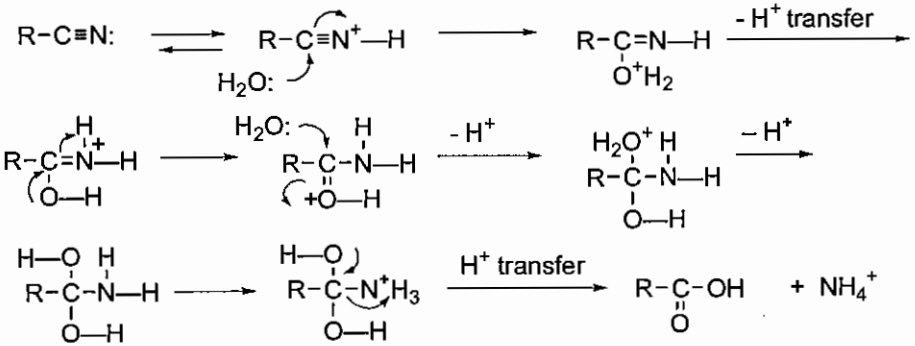


Dehydration of amides

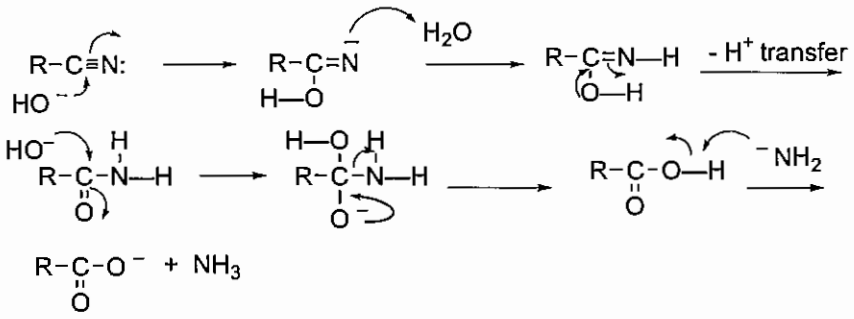


Reactions: Hydrolysis

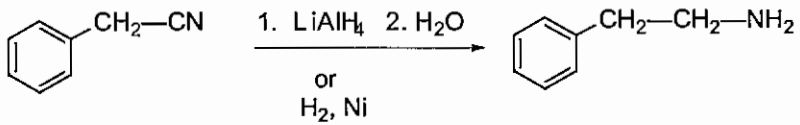
Acid



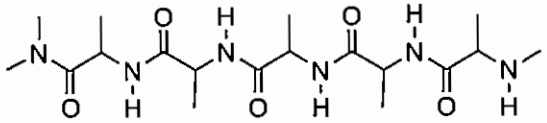
Base



Reduction

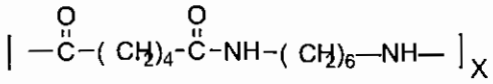


proteins



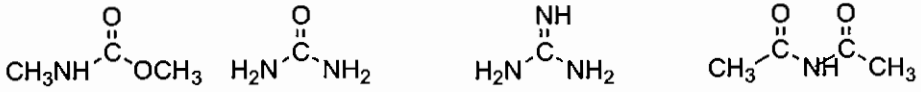
Polyamides

x $HO_2C(CH_2)_4CO_2H$ + X $H_2N(CH_2)_6NH_2$ ⟶



nylon 66

Compounds related to amides



carbamate urea guanidine imide

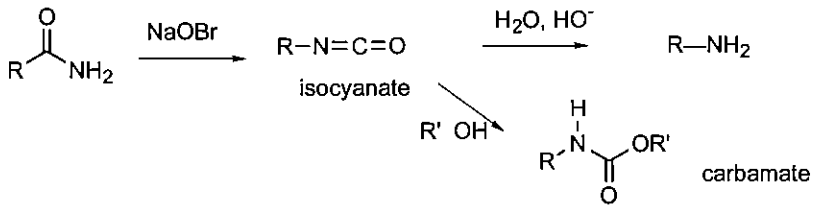
Spectra

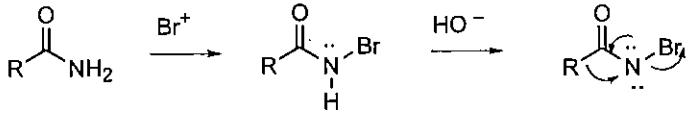
α-hydrogens in ^1H NMR

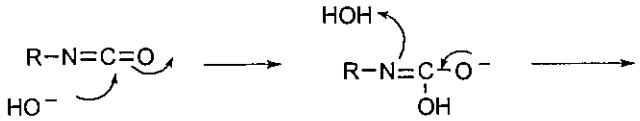
$CH_3-C\equiv N$	$CH_3-C(=O)-OCH_3$	$CH_3-C(=O)-NH_2$	$CH_3-C(=O)-OH$	$CH_3-C(=O)-Cl$
2.00	2.03	2.08	2.10	2.67

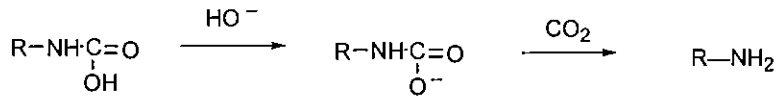
$R-C(=O)-Cl$	C=O stretch about 1800 cm^{-1}
$R-C(=O)-OCH_3$	C=O stretch about 1740 cm^{-1}; C—O 1200
$R-C(=O)-OCOR$	C=O stretch doublet; C—O 1100
$R-C(=O)-NH_2$	C=O stretch about 1700 cm^{-1}; NH bend 1515 - 1670; NH doublet 3500
$R-C(=O)-NHR$	C=O stretch about 1700 cm^{-1}; NH bend 1515 - 1670; NH 3300
$R-C(=O)-NR_2$	C=O stretch about 1700 cm^{-1}; no NH bend; no NH stretch
$-C\equiv N$	2200 cm-1

Hoffmann Rearrangement

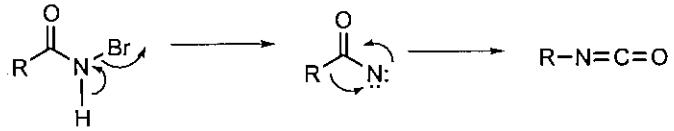


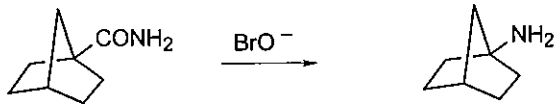






or via the nitrene

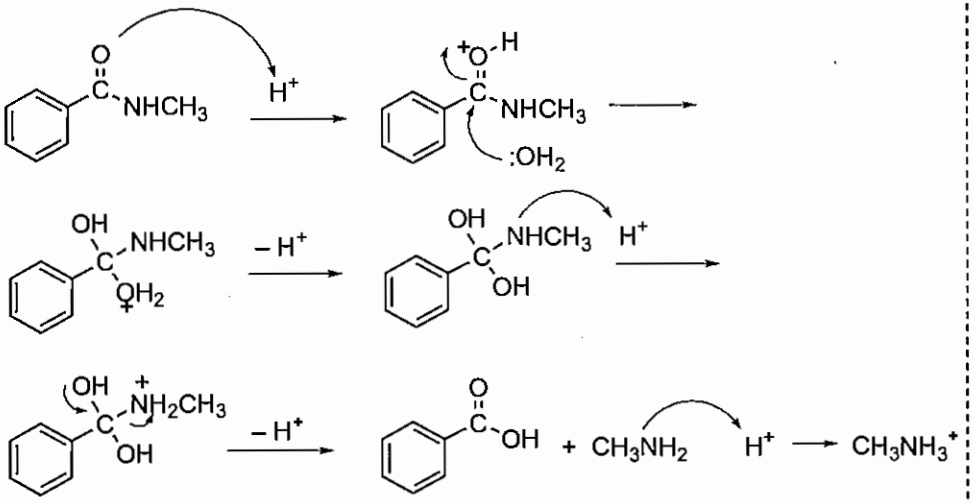




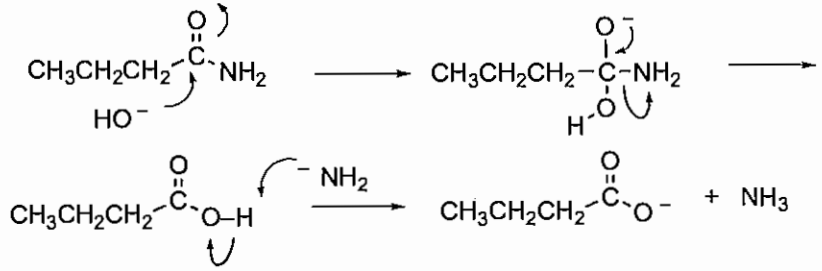
Reactions

Acid Hydrolysis

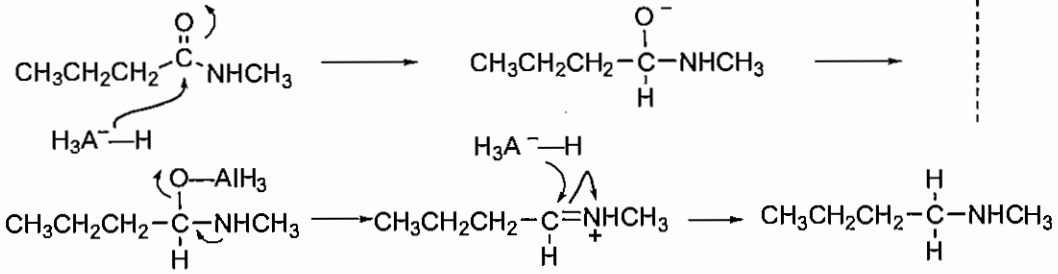
Not reversible since amine forms ammonium salt

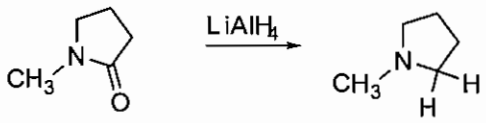


Base Hydrolysis

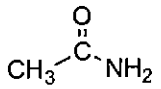


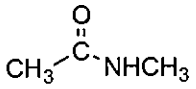
Reduction

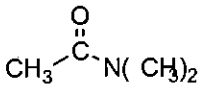




Amides


acetamide


N-methylacetamide

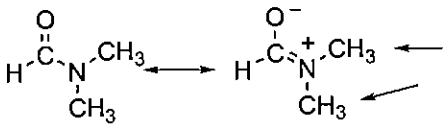

N,N-dimethylacetamide

Amides are not as basic as amines due to the overlap of the lone pair on nitrogen with the carbonyl pi bond.

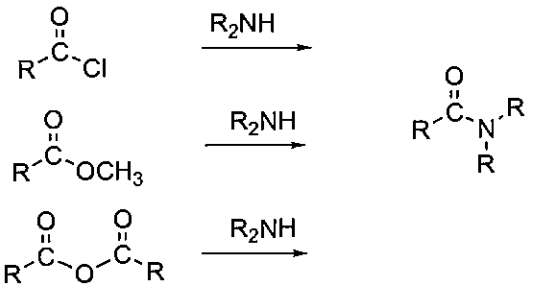
Amide pKb' s: 15 - 16; $C_6H_5NH_2$: pKb: 3.34.

The result is a partial double bond between the nitrogen and the carbonyl carbon. The barrier to rotation is about 18 kcal/ mol

This is evident from the difference in chemical shifts of the two methyl groups in dimethyl formamide in the 1H NMR.


different chemical shifts due to restricted rotation

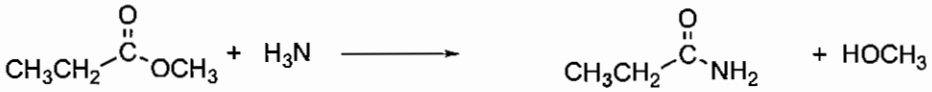
Preparation



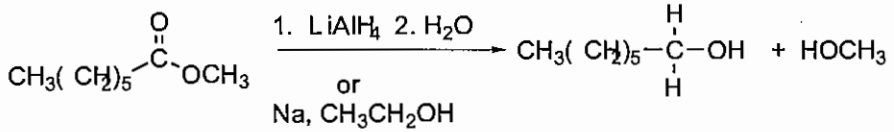
Reactions
Acid Hydrolysis

Not reversible since amine forms ammonium salt

Reaction with Ammonia



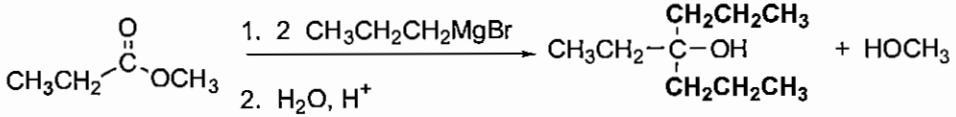
Reduction

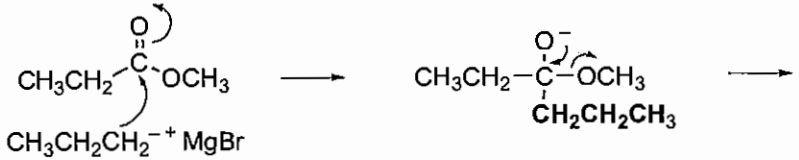


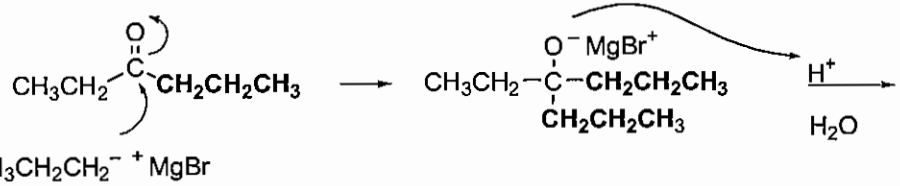
reduction always produces one primary alcohol plus the alcohol from the ether linkage of the ester

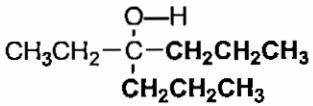
Reaction with Grignard reagents

Preparation of tertiary alcohols with at least two identical R groups







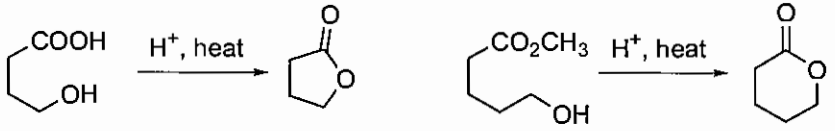


Addition to the ketone is faster than addition to the ester and therefore two additions occur

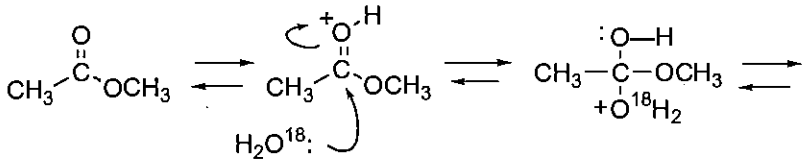
A secondary alcohol results from addition to a formate HCO_2R since hydrogen was already bonded to the carbonyl carbon.

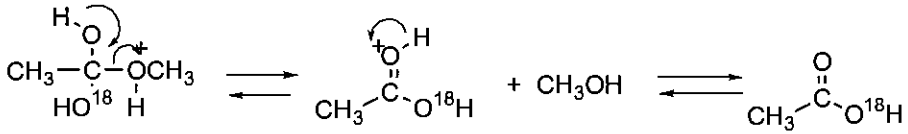
Lactones

Lactones are cyclic esters and react like esters. They are formed from acyclic hydroxy acids or hydroxy esters



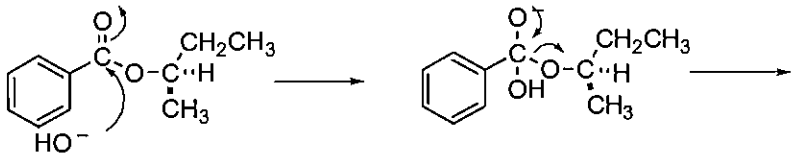
Acid hydrolysis: the reverse of esterification

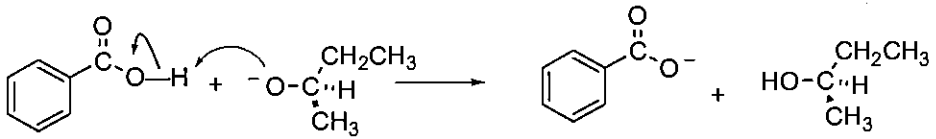




labelled water results in labelled carboxylic acid; no label in the alcohol

Base Hydrolysis

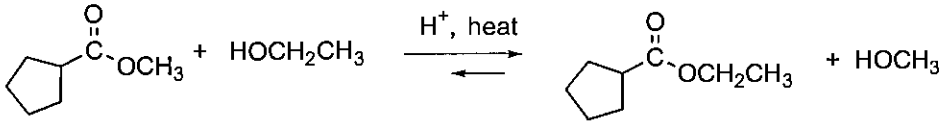




The product of the base hydrolysis is the carboxylate salt and the reaction is irreversible.
I f the alcohol is chiral; retention of configuration is observed.
Thus the C—O bond of the ester is broken, not the C—O bond of the alcohol.

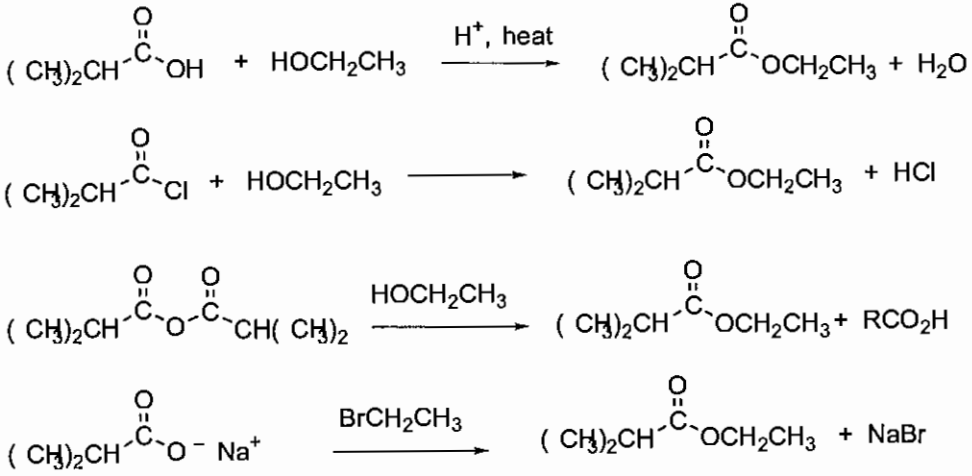
Transesterification

one alcohol is used in excess to drive the eq uilibrium in the desired direction



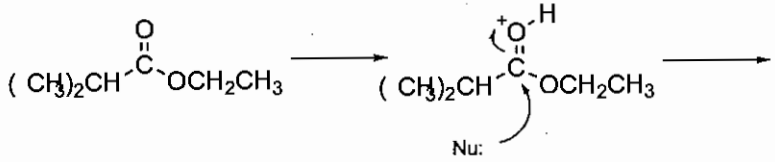
Esters

Preparation

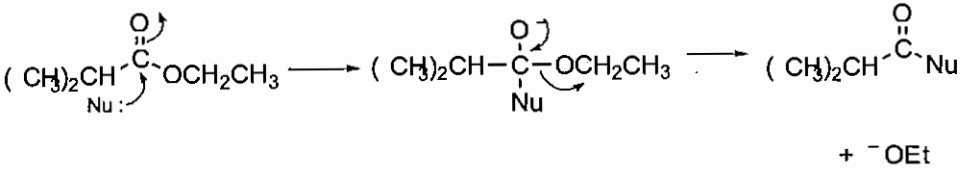


Reactions of Esters

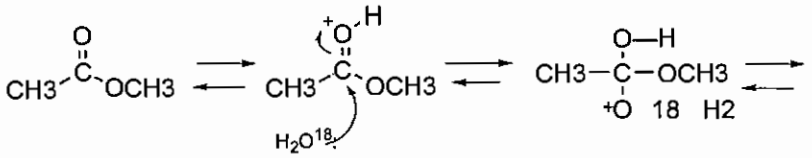
Acid can protonate the carbonyl oxygen and make the carbonyl carbon more susceptible to attack by nucleophiles

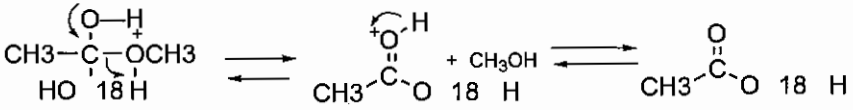


I n alkaline solution strong nucleophiles can effect addition-elimination



Acid hydrolysis: the reverse of esterification



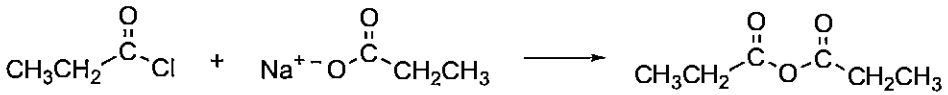


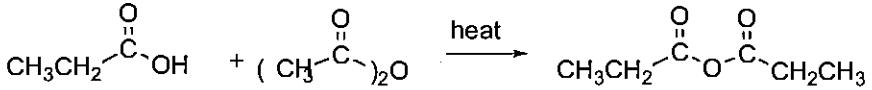
labelled water results in labelled carboxylic acid; no label in the alcohol

Base Hydrolysis

Anhydrides

Preparation

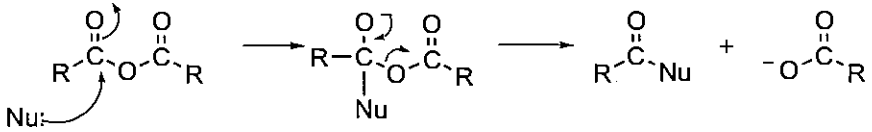




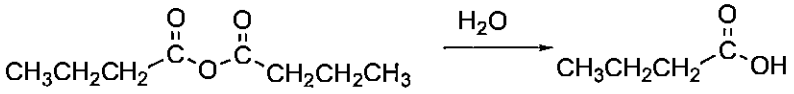
+ CH_3CO_2H distilled off to drive the equilibrium

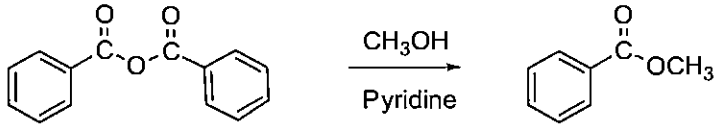
Reactions

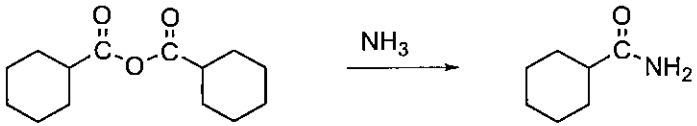
Same reactions as acid chlorides but with somewhat slower rates due to the poorer leaving group ability of RCO_2^-



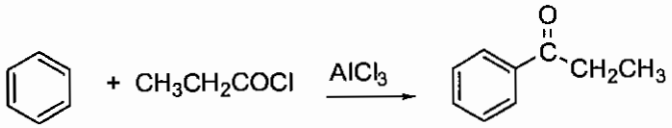
Nucleophiles: H_2O, ROH, ArOH, NH_3, RNH_2, R_2NH



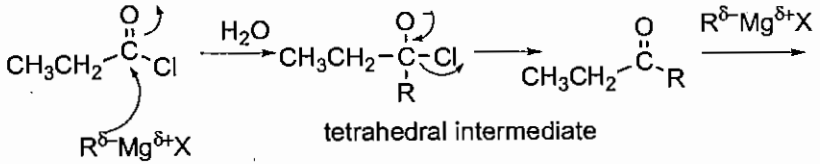


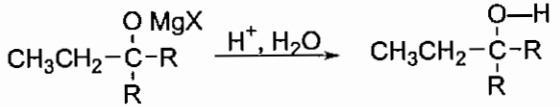


Fredel Crafts Reactions

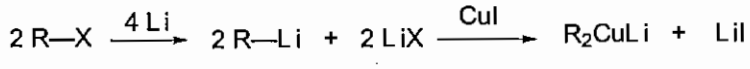


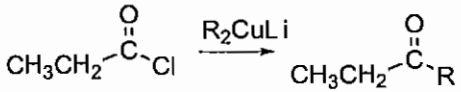
Reaction with Grignard Reagents

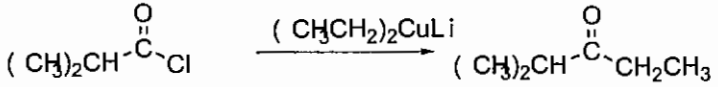




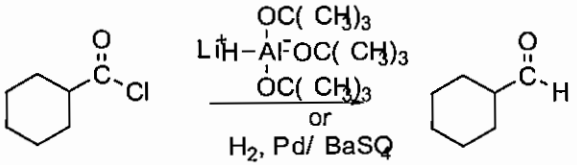
Reaction with Lithium Dialkyl Cuprates







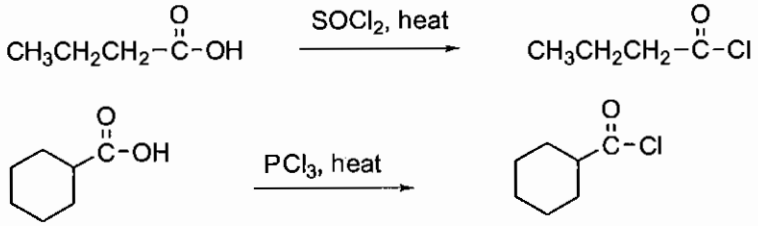
Reduction



$LiAl[OC(CH_3)_3]_3$ is less reactive than $LiAlH_4$ due to steric hindrance and the electron withdrawing effects of the oxygens

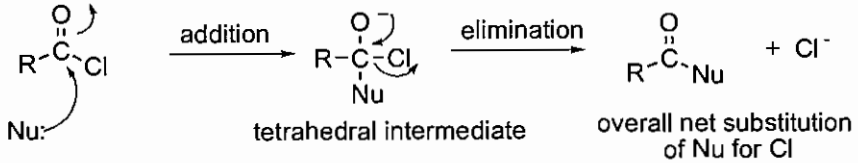
Acid Halides

Preparation



Reactions of Acid Halides

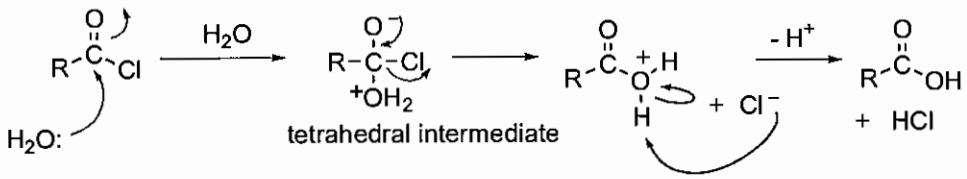
most reactive of the carboxylic acid derivatives since the halide ion is a good leaving group.



tetrahedral intermediate

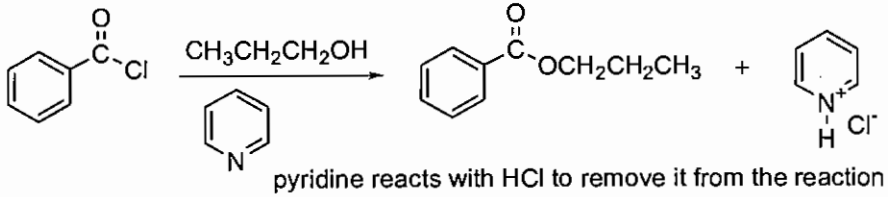
overall net substitution of Nu for Cl

Hydrolysis



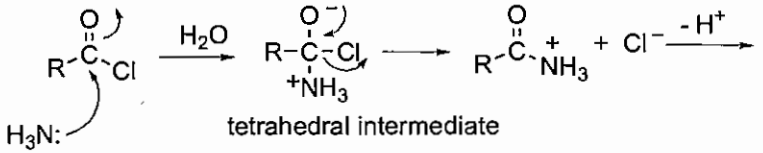
Rate decreases with increasing size of R since water solubility decreases

Ester formation

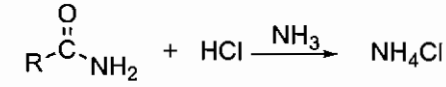


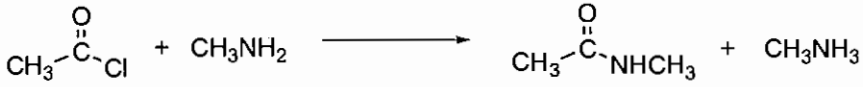
pyridine reacts with HCl to remove it from the reaction

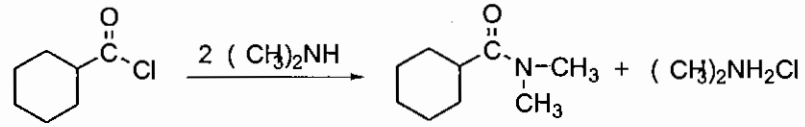
Amide formation



tetrahedral intermediate



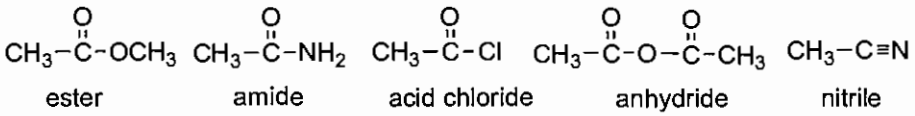




Use of an added tertiary amine avoids the loss of a second equivalent of nucleophilic amine

Derivatives of Carboxylic Acids

Any compound which yields a carboxylic acid on hydrolysis (acid or base) with water



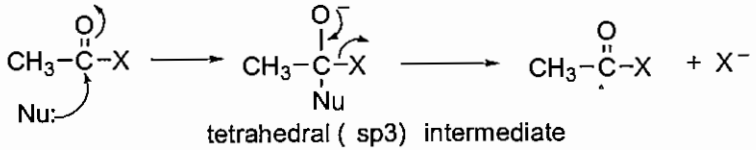
Reactivity

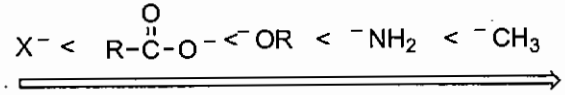
X = OR, Cl, NH_2, OCOR leaving groups
X = H, R, Ar not leaving groups

Aldehydes and ketones undergo nucleophilic addition

Carboxylic acid derivatives undergo nucleophilic substitution due to the presence of a leaving group on the carbonyl carbon

Nucleophilic acyl substitution





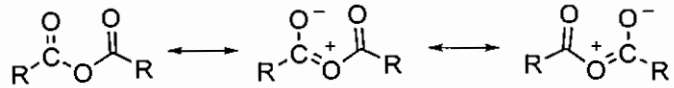
increasing basicity of acyl substituent (leaving group)

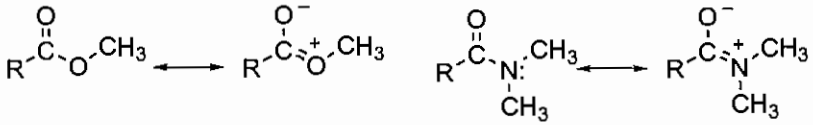
decreasing reactivity (leaving group ability)

Reactivity of carboxylc acid derivatives decreases with increasing basicity of leaving group

Acid chlorides and anhydrides react readily with water while esters and amides are fairly stable toward water and req uire acid or base to effect hydrolysis

Reacticvity is also related to the resonance donating ablity of the acyl substituent



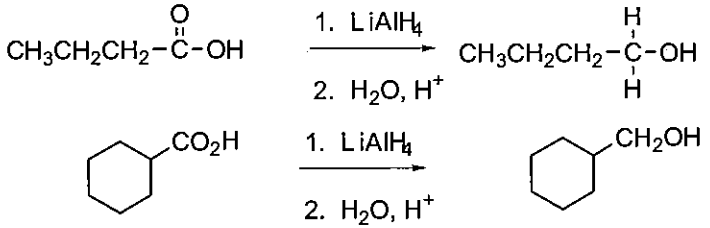


best resonance donor since nitrogen is least electronegative and better L ewis base:

Amides have significant C= N double bond character and hindered rotation about the N–Cacrbonyl bond.

Reduction of Carboxylic Acids

carboxylic acids can be reduced to primary alcohhols with LiAlH4



Polyfunctional Carboxylic Acids

Dicarboxylic acids are called dibasic or diprotic acids

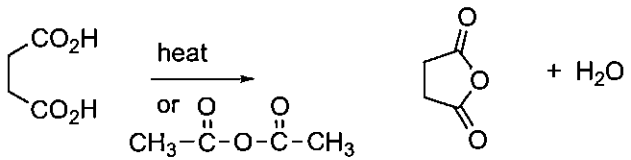
the acidity of the first COOH to lose a proton is increased by the electron withdrawing ability of the other COOH, but the acidity of the second is lower (pKa increased) because of the adj acent negative charge created by the first COO-

acid structure		pKa 1	2
oxalic acid	$HO_2C—CO_2H$	1.2	4.2
malonic acid	$HO_2C—CH_2—CO_2H$	2.8	5.7
succinic acid	$HO_2C—(CH_2)_2—CO_2H$	4.2	5.6
glutaric acid	$HO_2C—(CH_2)_3—CO_2H$	4.3	5.4
adipic acid H	$HO_2C—(CH_2)_4—CO_2H$	4.4	5.4

difference between pKa1 and pKa 2 decreses as the length of the chain increases since induction is directly dependent on distance

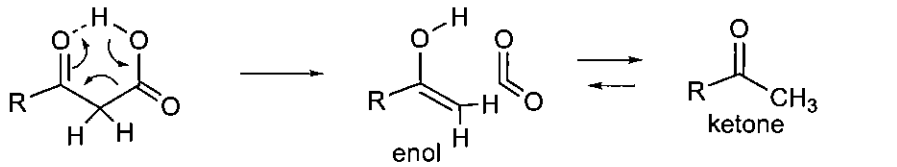
Anhydride formation

if a 5 or 6 membered ring can form, dicarboxylic acids form cyclic anhydrides with loss of water upon heating

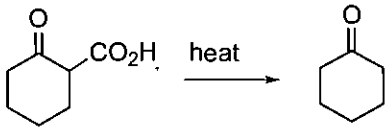


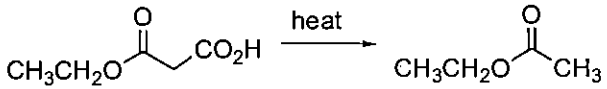
Decarboxylation

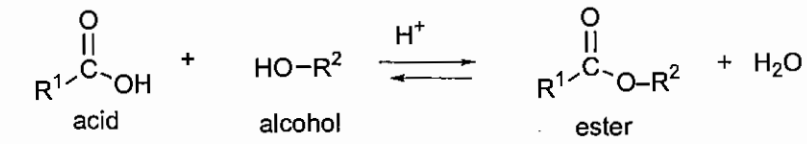
b-keto acids lose CO_2 (decarboxylate) on heating

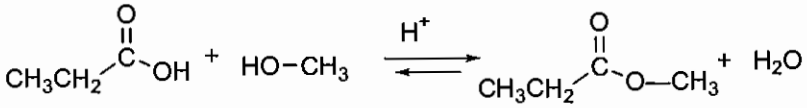


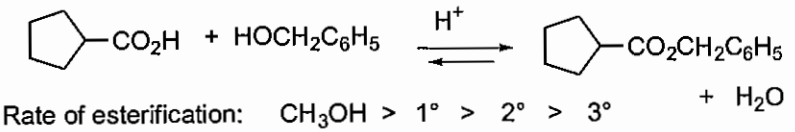
R = CH_3, alkyl, OH, OR









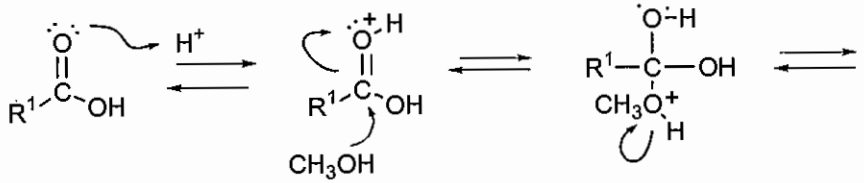


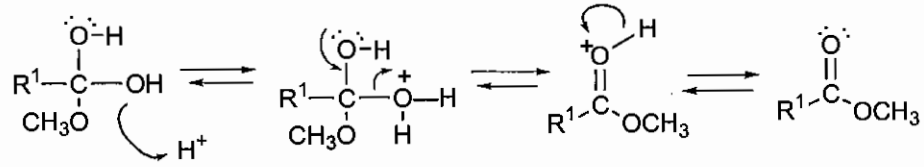
Rate of esterification: CH_3OH > 1° > 2° > 3°

R_3CCO_2H < R_2CHCO_2H < RCH_2CO_2H < CH_3CO_2H < HCO_2H

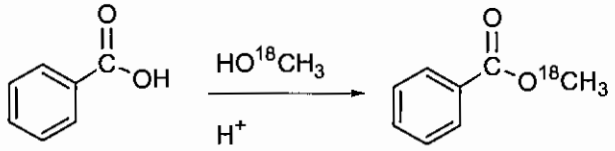
steric hindrance controls the rate of the reaction
Estierification proceeds through a series of reversible steps involving protonation and deprotonation

Mechanism of the esterification reaction:

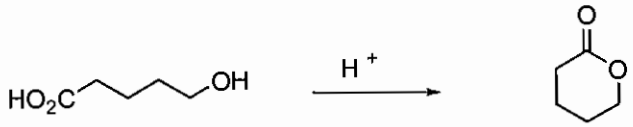




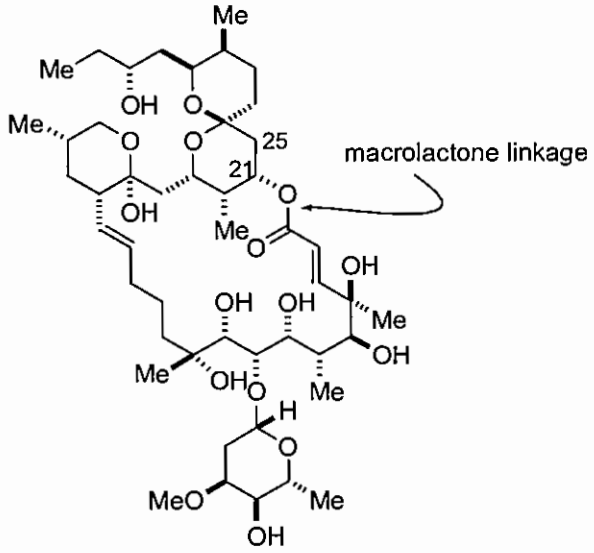
C—O bond of the acid is broken, not the C—O bond of the alcohol that is, the alcohol oxygen is incorporated into the ester not the oxygen from the acid —OH.



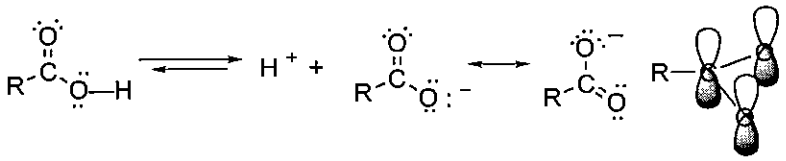
intramolecular ester are called lactones
formation of five and six membered lactones is very fast: yielding stable esters

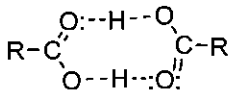


Many naturally occurring macrolactones are known and many have important biological activities such as the antibiotic cytovarycin



Carboxylic acids contain both a carbonyl and a hydroxyl function





carboxylic acid dimer:

results in higher melting and boiling points

Spectral Properties

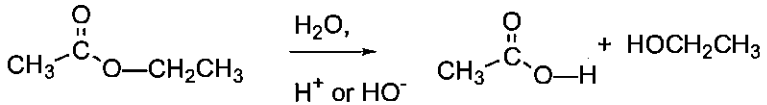
—OH stretch in the infrared is intense due to dimers...3300 - 3000
C=O stretch 1700 - 1725 shifted to 1680 - 1700 if conjugated

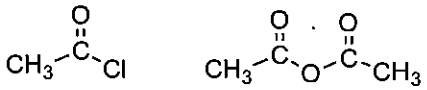
—COOH in 1H NMR at about 10 - 13 ppm as a broad singlet

Preparation

1. Hydrolysis of carboxylic acid derivatives
2. oxidation of alcohols, aldehydes, or alkenes
3. Grignard reactions

Hydrolysis of Carboxylic acid derivatives



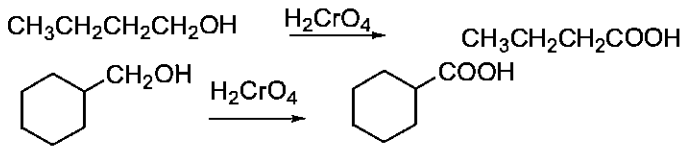


$CH_3C{\equiv}N$

also yield acetic acid on hydrolysis

Oxidation

alcohols



Simple Solubility Rules

1. All salts of the alkali (IA) metals are soluble.

2. All ammonium (NH_4^+) salts are soluble.

3. All salts containing the anions, NO_3^-, ClO_3^-, ClO_4^-, and $CH_3CO_2^-$ (nitrate, chlorate, perchlorate, and acetate) are soluble (except that $AgCH_3CO_2$ and $KClO_4$ are slightly soluble).

4. All chlorides, bromides, and iodides (Cl^-, Br^-, I^-) are soluble except those of Ag^+, Pb^{2+}, and Hg_2^{2+}. Note that $PbCl_2$ is slightly soluble.

5. All sulfates (SO_4^{2-}) are soluble except those of Pb^{2+}, Sr^{2+}, and Ba^{2+}. The sulfates of Ca^{2+} and Ag^+ are slightly soluble.

6. All metal oxides (O^{2-}) except those of the alkali (IA) metals and Ca^{2+}, Sr^{2+}, and Ba^{2+}, are insoluble. Metal oxides, when they do dissolve, react with water to form hydroxides (OH^-), as for example:

$$CaO(s) + H_2O(l) \rightarrow Ca^{+2}(aq) + 2OH^-(aq)$$

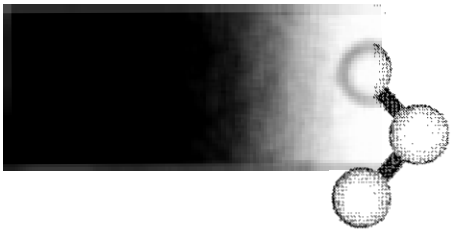
7. All hydroxides (OH^-) are insoluble except those of the alkali (IA) metals, and Ba^{2+} and Sr^{2+}. $Ca(OH)_2$ is slightly soluble.

8. All carbonates (CO_3^{2-}), phosphates (PO_4^{3-}), sulfides (S^{2-}), and sulfites (SO_3^{2-}) are insoluble except those of NH_4^+ and the alkali metals.

COMMON CATIONS AND ANIONS

Name of Cation	Formula
aluminum	Al^{3+}
ammonium	NH_4^+
barium	Ba^{2+}
bismuth	Bi^{3+}
cadmium	Cd^{2+}
calcium	Ca^{2+}
chromium(III).(chromic)	Cr^{3+}
cobalt(II)....(cobaltous).	Co^{2+}
copper(II)....(cupric)....	Cu^{2+}
hydrogen.....(proton)....	H^+
hydronium.....(oxonium)...	H_3O^+
iron(II).....(ferrous)...	Fe^{2+}
iron(III).....(ferric)....	Fe^{3+}
lead(II).....(plumbous)..	Pb^{2+}
magnesium	Mg^{2+}
manganese(II).(manganous).	Mn^{2+}
mercury(II)...(mercuric)..	Hg^{2+}
mercury(I)...(mercurous)..	Hg_2^{2+}
nickel(II)....(nickelous).	Ni^{2+}
copper(I).....(cuprous)...	Cu^+
potassium	K^+
silver(I).....(argentous).	Ag^+
sodium	Na^+
tin(IV).....(stannic)...	Sn^{4+}
tin(II).....(stannous)..	Sn^{2+}
zinc	Zn^{2+}

Name of Anion	Formula
acetate	$CH_3CO_2^-$
(or OAc^- or $C_2H_3O_2^-$)	
bromide	Br^-
carbonate	CO_3^{2-}
chlorate	ClO_3^-
chloride	Cl^-
chlorite	ClO_2^-
chromate	CrO_4^{2-}
cyanide	CN^-
dichromate	$Cr_2O_7^{2-}$
fluoride	F^-
hydrogen carbonate	HCO_3^-
(or bicarbonate)	
hydrogen sulfate	HSO_4^-
(or bisulfate)	
hydroxide	OH^-
hypochlorite	ClO^-
iodide	I^-
nitrate	NO_3^-
nitrite	NO_2^-
orthophosphate	PO_4^{3-}
oxalate	$C_2O_4^{2-}$
oxide	O^{2-}
perchlorate	ClO_4^-
permanganate	MnO_4^-
sulfate	SO_4^{2-}
sulfide	S^{2-}
thiocyanate	SCN^-
thiosulfate	$S_2O_3^{2-}$



Appendix

Solubility Products

Bromide (Br^-)	pK_{sp}	K_{sp}
CuBr	8.3	5×10^{-9}
AgBr	12.30	5.0×10^{-13}
Hg_2Br_2	22.25	5.6×10^{-23}
$HgBr_2$(μ = 0.5 M)	18.9	1.3×10^{-19}
$PbBr_2$(μ = 4.0 M)	5.68	2.1×10^{-6}

Carbonate (CO_3^{2-})	pK_{sp}	K_{sp}
$MgCO_3$	7.46	3.5×10^{-8}
$CaCO_3$ (calcite)	8.35	4.5×10^{-9}
$CaCO_3$ (aragonite)	8.22	6.0×10^{-9}
$SrCO_3$	9.03	9.3×10^{-10}
$BaCO_3$	8.30	5.0×10^{-9}
$MnCO_3$	9.30	5.0×10^{-10}
$FeCO_3$	10.68	2.1×10^{-11}
$CoCO_3$	9.98	1.0×10^{-10}
$NiCO_3$	6.87	1.3×10^{-7}
Ag_2CO_3	11.09	8.1×10^{-12}
Hg_2CO_3	16.05	8.9×10^{-17}
$ZnCO_3$	10.00	1.0×10^{-10}
$CdCO_3$	13.74	1.8×10^{-14}
$PbCO_3$	13.13	7.4×10^{-14}

Chloride (Cl^-)	pK_{sp}	K_{sp}
CuCl	6.73	1.9×10^{-7}
AgCl	9.74	1.8×10^{-10}
Hg_2Cl_2	17.91	1.2×10^{-18}
$PbCl_2$	4.78	1.7×10^{-5}

Chromate (CrO_4^{2-})	pK_{sp}	K_{sp}
$BaCrO_4$	9.67	2.1×10^{-10}
$CuCrO_4$	5.44	3.6×10^{-6}
Ag_2CrO_4	11.92	1.2×10^{-12}
Hg_2CrO_4	8.70	2.0×10^{-9}

Cyanide (CN^-)	pK_{sp}	K_{sp}
AgCN	15.66	2.2×10^{-16}
$Zn(CN)_2$ (μ = 3.0 M)	15.5	3×10^{-16}
$Hg_2(CN)_2$	39.3	5×10^{-40}

Ferrocyanide ($[Fe(CN)_6^{4-}]$)	pK_{sp}	K_{sp}
$Zn_2[Fe(CN)_6]$	15.68	2.1×10^{-16}
$Cd_2[Fe(CN)_6]$	17.38	4.2×10^{-18}
$Pb_2[Fe(CN)_6]$	18.02	9.5×10^{-19}

Fluoride (F^-)	pK_{sp}	K_{sp}
MgF_2	8.18	6.6×10^{-9}
CaF_2	10.41	3.9×10^{-11}
SrF_2	8.54	2.9×10^{-9}
BaF_2	5.76	1.7×10^{-6}
PbF_2	7.44	3.6×10^{-8}

Hydroxide (OH^-)	pK_{sp}	K_{sp}
$Mg(OH)_2$	11.15	7.1×10^{-12}
$Ca(OH)_2$	5.19	6.5×10^{-6}
$Ba(OH)_2 \cdot 8H_2O$	3.6	3×10^{-4}
$La(OH)_3$	20.7	2×10^{-21}
$Mn(OH)_2$	12.8	1.6×10^{-13}
$Fe(OH)_2$	15.1	8×10^{-16}
$Co(OH)_2$	14.9	1.3×10^{-15}
$Ni(OH)_2$	15.2	6×10^{-16}
$Cu(OH)_2$	19.32	4.8×10^{-20}
$Fe(OH)_3$	38.8	1.6×10^{-39}
$Co(OH)_3$ (T = 19 °C)	44.5	3×10^{-45}
Ag_2O ($+H_2O \rightleftharpoons 2Ag^+ + 2OH^-$)	15.42	3.8×10^{-16}
Cu_2O ($+ H_2O \rightleftharpoons 2Cu^+ + 2OH^-$)	29.4	4×10^{-30}
$Zn(OH)_2$ (amorphous)	15.52	3.0×10^{-16}
$Cd(OH)_2$ (β)	14.35	4.5×10^{-15}

continued

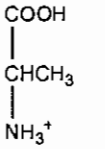
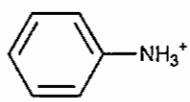
Solubility Products—*continued*

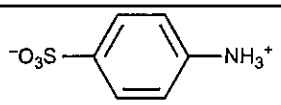
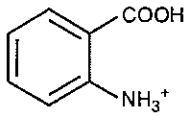
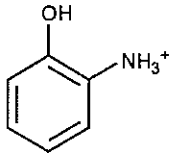
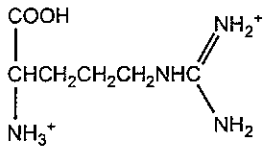
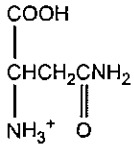
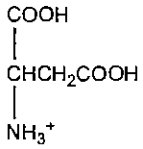
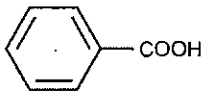
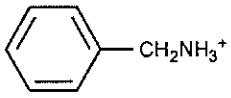
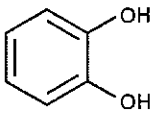
Hydroxide (OH^-)	pK_{sp}	K_{sp}
HgO (red) (+ $H_2O \rightleftharpoons Hg^{2+} + 2OH^-$)	25.44	3.6×10^{-26}
SnO (+ $H_2O \rightleftharpoons Sn^{2+} + 2OH^-$)	26.2	6×10^{-27}
PbO (yellow) (+ $H_2O \rightleftharpoons Pb^{2+} + 2OH^-$)	15.1	8×10^{-16}
$Al(OH)_3$ (α)	33.5	3×10^{-34}
Iodate (IO_3^-)	**pK_{sp}**	**K_{sp}**
$Ca(IO_3)_2$	6.15	7.1×10^{-7}
$Ba(IO_3)_2$	8.81	1.5×10^{-9}
$AgIO_3$	7.51	3.1×10^{-8}
$Hg_2(IO_3)_2$	17.89	1.3×10^{-18}
$Zn(IO_3)_2$	5.41	3.9×10^{-6}
$Cd(IO_3)_2$	7.64	2.3×10^{-8}
$Pb(IO_3)_2$	12.61	2.5×10^{-13}
Iodide (I^-)	**pK_{sp}**	**K_{sp}**
AgI	16.08	8.3×10^{-17}
Hg_2I_2	28.33	4.7×10^{-29}
HgI_2 ($\mu = 0.5$ M)	27.95	1.1×10^{-28}
PbI_2	8.10	7.9×10^{-9}
Oxalate ($C_2O_4^{2-}$)	**pK_{sp}**	**K_{sp}**
CaC_2O_4 ($\mu = 0.1$ M, $T = 20$ °C)	7.9	1.3×10^{-8}
BaC_2O_4 ($\mu = 0.1$ M, $T = 20$ °C)	6.0	1×10^{-6}
SrC_2O_4 ($\mu = 0.1$ M, $T = 20$ °C)	6.4	4×10^{-7}
Phosphate (PO_4^{3-})	**pK_{sp}**	**K_{sp}**
$Fe_3(PO_4)_2 \cdot 8H_2O$	36.0	1×10^{-36}
$Zn_3(PO_4)_2 \cdot 4H_2O$	35.3	5×10^{-36}
Ag_3PO_4	17.55	2.8×10^{-18}
$Pb_3(PO_4)_2$ ($T = 38$ °C)	43.53	3.0×10^{-44}

Sulfate (SO_4^{2-})	pK_{sp}	K_{sp}
$CaSO_4$	4.62	2.4×10^{-5}
$SrSO_4$	6.50	3.2×10^{-7}
$BaSO_4$	9.96	1.1×10^{-10}
Ag_2SO_4	4.83	1.5×10^{-5}
Hg_2SO_4	6.13	7.4×10^{-7}
$PbSO_4$	7.79	1.6×10^{-8}
Sulfide (S^{2-})	**pK_{sp}**	**K_{sp}**
MnS (green)	13.5	3×10^{-14}
FeS	18.1	8×10^{-19}
CoS (β)	25.6	3×10^{-26}
NiS (γ)	26.6	3×10^{-27}
CuS	36.1	8×10^{-37}
Cu_2S	48.5	3×10^{-49}
Ag_2S	50.1	8×10^{-51}
ZnS (α)	24.7	2×10^{-25}
CdS	27.0	1×10^{-27}
Hg_2S (red)	53.3	5×10^{-54}
PbS	27.5	3×10^{-28}
Thiocyanate (SCN^-)	**pK_{sp}**	**K_{sp}**
CuSCN ($\mu = 5.0$ M)	13.40	4.0×10^{-14}
AgSCN	11.97	1.1×10^{-12}
$Hg_2(SCN)_2$	19.52	3.0×10^{-20}
$Hg(SCN)_2$ ($\mu = 1.0$ M)	19.56	2.8×10^{-20}

Source: All values are from Martell, A. E.; Smith, R. M. *Critical Stability Constants*, Vol. 4. Plenum Press: New York, 1976. Unless otherwise stated, values are for 25 °C and zero ionic strength.

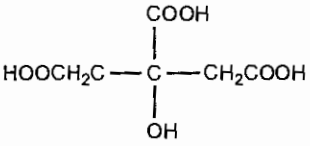
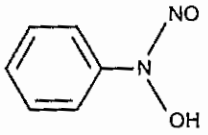
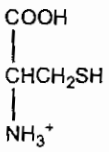
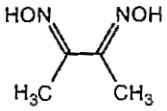
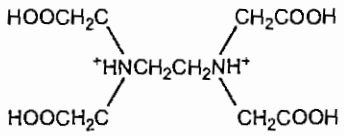
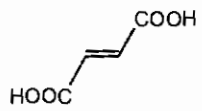
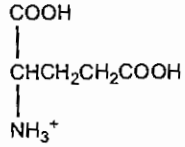
Acid Dissociation Constants

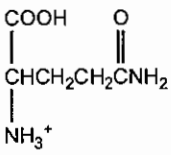
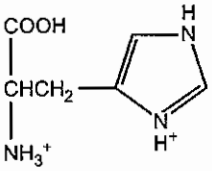
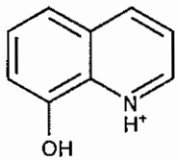
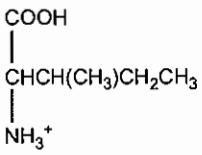
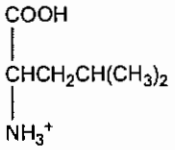
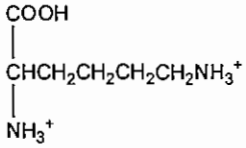
Compound	Conjugate Acid	pK_a	K_a
acetic acid	CH_3COOH	4.757	1.75×10^{-5}
adipic acid	$HOOC(CH_2)_4COOH$	4.42	3.8×10^{-5}
		5.42	3.8×10^{-6}
alanine		2.348 (COOH)	4.49×10^{-3}
		9.867 (NH_3)	1.36×10^{-10}
aminobenzene		4.601	2.51×10^{-5}

Compound	Conjugate Acid	pK_a	K_a
4-aminobenzene sulfonic acid		3.232	5.86×10^{-4}
2-aminobenzoic acid		2.08 (COOH) 4.96 (NH_3)	8.3×10^{-3} 1.1×10^{-5}
2-aminophenol		4.78 (NH_3); (T = 20 °C) 9.97 (OH); (T = 20 °C)	1.7×10^{-5} 1.05×10^{-10}
ammonia	NH_4^+	9.244	5.70×10^{-10}
arginine		1.823 (COOH) 8.991 (NH_3) (12.48) (NH_2)	1.50×10^{-2} 1.02×10^{-9} 3.3×10^{-13}
arsenic acid	H_3AsO_4	2.24 6.96 11.50	5.8×10^{-3} 1.1×10^{-7} 3.2×10^{-12}
asparagine		2.14 (COOH); (μ = 0.1 M) 8.72 (NH_3); (μ = 0.1 M)	7.2×10^{-3} 1.9×10^{-9}
aspartic acid		1.990 (α-COOH) 3.900 (β-COOH) 10.002 (NH_3)	1.02×10^{-2} 1.26×10^{-4} 9.95×10^{-11}
benzoic acid		4.202	6.28×10^{-5}
benzylamine		9.35	4.5×10^{-10}
boric acid	H_3BO_3	9.236 (12.74); (T = 20 °C) (13.80); (T = 20 °C)	5.81×10^{-10} 1.82×10^{-13} 1.58×10^{-14}
carbonic acid	H_2CO_3	6.352 10.329	4.45×10^{-7} 4.69×10^{-11}
catechol		9.40 12.8	4.0×10^{-10} 1.6×10^{-13}

continued

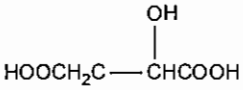
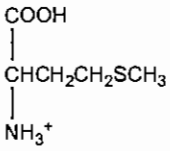
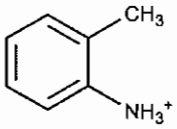
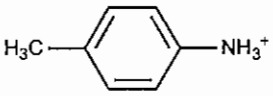
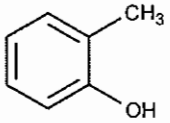
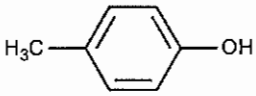
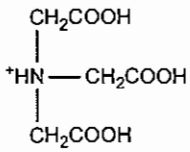
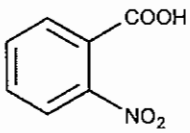
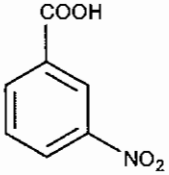
Acid Dissociation Constants—*continued*

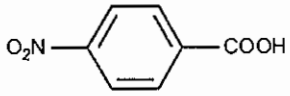
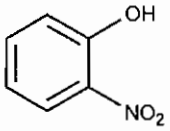
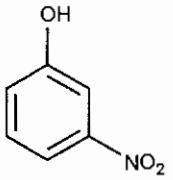
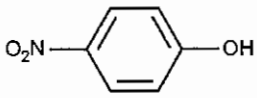
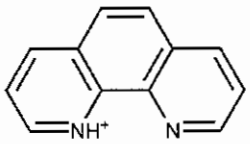
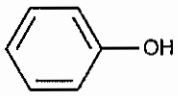
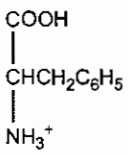
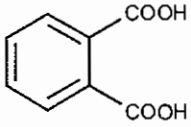
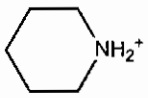
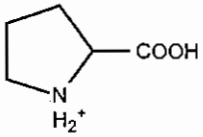
Compound	Conjugate Acid	pK_a	K_a
chloroacetic acid	$ClCH_2COOH$	2.865	1.36×10^{-3}
chromic acid	H_2CrO_4	–0.2; (T = 20 °C)	1.6
		6.51	3.1×10^{-7}
citric acid		3.128 (COOH)	7.45×10^{-4}
		4.761 (COOH)	1.73×10^{-5}
		6.396 (COOH)	4.02×10^{-7}
cupferron		4.16; (μ = 0.1 M)	6.9×10^{-5}
cysteine		(1.71) (COOH)	1.9×10^{-2}
		8.36 (SH)	4.4×10^{-9}
		10.77 (NH_3)	1.7×10^{-11}
dichloroacetic acid	$Cl_2CHCOOH$	1.30	5.0×10^{-2}
diethylamine	$(CH_3CH_2)_2NH_2^+$	10.933	1.17×10^{-11}
dimethylamine	$(CH_3)_2NH_2^+$	10.774	1.68×10^{-11}
dimethylgloxime		10.66	2.2×10^{-11}
		12.0	1×10^{-12}
ethylamine	$CH_3CH_2NH_3^+$	10.636	2.31×10^{-11}
ethylenediamine	$^+H_3NCH_2CH_2NH_3^+$	6.848	1.42×10^{-7}
		9.928	1.18×10^{-10}
ethylenediaminetetraacetic acid (EDTA)		0.0 (COOH); (μ = 1.0 M)	1.0
		1.5 (COOH); (μ = 0.1 M)	3.2×10^{-2}
		2.0 (COOH); (μ = 0.1 M)	1.0×10^{-2}
		2.68 (COOH); (μ = 0.1 M)	2.1×10^{-3}
		6.11 (NH); (μ = 0.1 M)	7.8×10^{-7}
		10.17 (NH); (μ = 0.1 M)	6.8×10^{-11}
formic acid	HCOOH	3.745	1.80×10^{-4}
fumaric acid		3.053	8.85×10^{-4}
		4.494	3.21×10^{-5}
glutamic acid		2.23 (α-COOH)	5.9×10^{-3}
		4.42 (λ-COOH)	3.8×10^{-5}
		9.95 (NH_3)	1.12×10^{-10}

Compound	Conjugate Acid	pK_a	K_a
glutamine		2.17 (COOH); (μ = 0.1 M) 9.01 (NH_3); (μ = 0.1 M)	6.8×10^{-3} 9.8×10^{-10}
glycine	$^+H_3NCH_2COOH$	2.350 (COOH) 9.778 (NH_3)	4.47×10^{-3} 1.67×10^{-10}
glycolic acid	$HOCH_2COOH$	3.831 (COOH)	1.48×10^{-4}
histidine		1.7 (COOH); (μ = 0.1 M) 6.02 (NH); (μ = 0.1 M) 9.08 (NH_3); (μ = 0.1 M)	2×10^{-2} 9.5×10^{-7} 8.3×10^{-10}
hydrogen cyanide	HCN	9.21	6.2×10^{-10}
hydrogen fluoride	HF	3.17	6.8×10^{-4}
hydrogen peroxide	H_2O_2	11.65	2.2×10^{-12}
hydrogen sulfide	H_25	7.02 13.9	9.5×10^{-8} 1.3×10^{-14}
hydrogen thiocyanate	HSCN	0.9	1.3×10^{-1}
8-hydroxyquinoline		4.91 (NH) 9.81 (OH)	1.23×10^{-5} 1.55×10^{-10}
hydroxylamine	$HONH_3^+$	5.96	1.1×10^{-6}
hypobromous	HOBr	8.63	2.3×10^{-9}
hypochlorous	HOCl	7.53	3.0×10^{-8}
hypoiodous	HOI	10.64	2.3×10^{-11}
iodic acid	HIO_3	0.77	1.7×10^{-1}
isoleucine		2.319 (COOH) 9.754 (NH_3)	4.80×10^{-3} 1.76×10^{-10}
leucine		2.329 (COOH) 9.747 (NH_3)	4.69×10^{-3} 1.79×10^{-10}
lysine		2.04 (COOH); (μ = 0.1 M) 9.08 (α-NH_3); (μ = 0.1 M) 10.69 (ε-NH_3);(μ = 0.1 M)	9.1×10^{-3} 8.3×10^{-10} 2.0×10^{-11}

continued

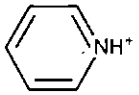
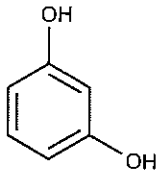
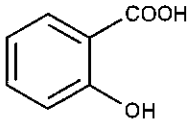
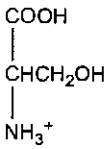
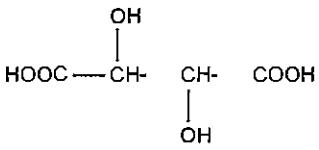
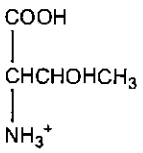
Acid Dissociation Constants—*continued*

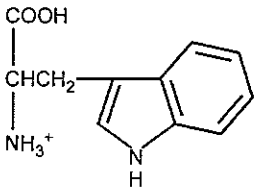
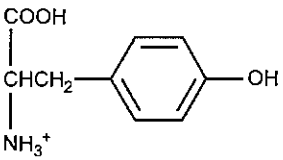
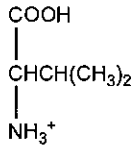
Compound	Conjugate Acid	pK_a	K_a
maleic acid		1.910 6.332	1.23×10^{-2} 4.66×10^{-7}
malic acid		3.459 (COOH) 5.097 (COOH)	3.48×10^{-4} 8.00×10^{-6}
malonic acid	$HOOCCH_2COOH$	2.847 5.696	1.42×10^{-3} 2.01×10^{-6}
methionine		2.20 (COOH); (μ = 0.1 M) 9.05 (NH_3); (μ = 0.1 M)	6.3×10^{-3} 8.9×10^{-10}
methylamine	$CH_3NH_3^+$	10.64	2.3×10^{-11}
2-methylaniline		4.447	3.57×10^{-5}
4-methylaniline		5.084	8.24×10^{-6}
2-methylphenol		10.28	5.2×10^{-11}
4-methylphenol		10.26	5.5×10^{-11}
nitrilotriacetic acid		1.1 (COOH); (T = 20 °C , μ = 1.0 M) 1.650 (COOH); (T = 20 °C) 2.940 (COOH); (T = 20 °C) 10.334 (NH_3); (T = 20 °C)	8×10^{-2} 2.24×10^{-2} 1.15×10^{-3} 4.63×10^{-11}
2-nitrobenzoic acid		2.179	6.62×10^{-3}
3-nitrobenzoic acid		3.449	3.56×10^{-4}

Compound	Conjugate Acid	pK_a	K_a
4-nitrobenzoic acid		3.442	3.61×10^{-4}
2-nitrophenol		7.21	6.2×10^{-8}
3-nitrophenol		8.39	4.1×10^{-9}
4-nitrophenol		7.15	7.1×10^{-8}
nitrous acid	HNO_2	3.15	7.1×10^{-4}
oxalic acid	$H_2C_2O_4$	1.252	5.60×10^{-2}
		4.266	5.42×10^{-5}
1,10-phenanthroline		4.86	1.38×10^{-5}
phenol		9.98	1.05×10^{-10}
phenylalanine		2.20 (COOH)	6.3×10^{-3}
		9.31 (NH_3)	4.9×10^{-10}
phosphoric acid	H_3PO_4	2.148	7.11×10^{-3}
		7.199	6.32×10^{-8}
		12.35	4.5×10^{-13}
phthalic acid		2.950	1.12×10^{-3}
		5.408	3.91×10^{-6}
piperidine		11.123	7.53×10^{-12}
proline		1.952 (COOH)	1.12×10^{-2}
		10.640 (NH)	2.29×10^{-11}

continued

Acid Dissociation Constants—*continued*

Compound	Conjugate Acid	pK_a	K_a
propanoic acid	CH_3CH_2COOH	4.874	1.34×10^{-5}
propylamine	$CH_3CH_2CH_2NH_3^+$	10.566	2.72×10^{-11}
pyridine		5.229	5.90×10^{-6}
resorcinol		9.30 11.06	5.0×10^{-10} 8.7×10^{-12}
salicylic acid		2.97 (COOH) 13.74 (OH)	1.07×10^{-3} 1.8×10^{-14}
serine		2.187 (COOH) 9.209 (NH_3)	6.50×10^{-3} 6.18×10^{-10}
succinic acid	$HOOCCH_2CH_2COOH$	4.207 5.636	6.21×10^{-5} 2.31×10^{-6}
sulfuric acid	H_2SO_4	strong 1.99	strong 1.0×10^{-2}
sulfurous acid	H_2SO_3	1.91 7.18	1.2×10^{-2} 6.6×10^{-8}
D-tartaric acid		3.036 (COOH) 4.366 (COOH)	9.20×10^{-4} 4.31×10^{-5}
threonine		2.088 (COOH) 9.100 (NH_3)	8.17×10^{-3} 7.94×10^{-10}
thiosulfuric acid	$H_2S_2O_3$	0.6 1.6	3×10^{-1} 3×10^{-2}
trichloroacetic acid	Cl_3CCOOH	0.66; ($\mu = 0.1$ M)	2.2×10^{-1}
triethanolamine	$(HOCH_2CH_2)_3NH^+$	7.762	1.73×10^{-8}
triethylamine	$(CH_3CH_2)_3NH^+$	10.715	1.93×10^{-11}

Compound	Conjugate Acid	pK_a	K_a
trimethylamine	$(CH_3)_3NH^+$	9.800	1.58×10^{-10}
tris(hydroxymethyl)-aminomethane (TRIS or THAM)	$(HOCH_2)_3CNH_3^+$	8.075	8.41×10^{-9}
tryptophan		2.35 (COOH); (μ = 0.1 M) 9.33 (NH_3); (μ = 0.1 M)	4.5×10^{-3} 4.7×10^{-10}
tyrosine		2.17 (COOH); (μ = 0.1 M) 9.19 (NH_3) 10.47 (OH)	6.8×10^{-3} 6.5×10^{-10} 3.4×10^{-11}
valine		2.286 (COOH) 9.718 (OH)	5.18×10^{-3} 1.91×10^{-10}

Source: All values are from Martell, A. E.; Smith, R. M. *Critical Stability Constants,* Vol. 1–4. Plenum Press: New York, 1976. Unless otherwise stated, values are for 25 °C and zero ionic strength. Values in parentheses are considered less reliable.

Metal–Ligand Formation Constants

Acetate CH_3COO^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Mg^{2+}	1.27					
Ca^{2+}	1.18					
Ba^{2+}	1.07					
Mn^{2+}	1.40					
Fe^{2+}	1.40					
Co^{2+}	1.46					
Ni^{2+}	1.43					
Cu^{2+}	2.22	1.41				
Ag^+	0.73	–0.09				
Zn^{2+}	1.57					
Cd^{2+}	1.93	1.22	–0.89			
Pb^{2+}	2.68	1.40				

continued

Metal–Ligand Formation Constants—*continued*

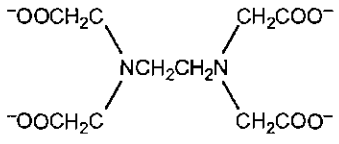
Ammonia NH_3	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Ag^+	3.31	3.91				
Co^{2+} (T = 20 °C)	1.99	1.51	0.93	0.64	0.06	–0.74
Ni^{2+}	2.72	2.17	1.66	1.12	0.67	–0.03
Cu^{2+}	4.04	3.43	2.80	1.48		
Zn^{2+}	2.21	2.29	2.36	2.03		
Cd^{2+}	2.55	2.01	1.34	0.84		

Chloride Cl^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Cu^{2+}	0.40					
Fe^{3+}	1.48	0.65				
Ag^+ (μ = 5.0 M)	3.70	1.92	0.78	–0.3		
Zn^{2+}	0.43	0.18	–0.11	–0.3		
Cd^{2+}	1.98	1.62	–0.2	–0.7		
Pb^{2+}	1.59	0.21	–0.1	–0.3		

Cyanide CN^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Fe^{2+}						35.4 (β_6)
Fe^{3+}						43.6 (β_6)
Ag^+		20.48 (β_2)	0.92			
Zn^{2+}		11.07 (β_2)	4.98	3.57		
Cd^{2+}	6.01	5.11	4.53	2.27		
Hg^{2+}	17.00	15.75	3.56	2.66		
Ni^{2+}				30.22 (β_4)		

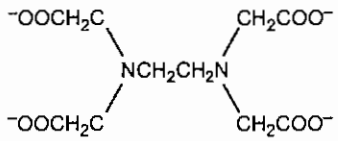
Ethylenediamine $H_2NCH_2CH_2NH_2$	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Ni^{2+}	7.38	6.18	4.11			
Cu^{2+}	10.48	9.07				
Ag^+ (T = 20 °C , μ = 0.1 M)	4.70	3.00				
Zn^{2+}	5.66	4.98	3.25			
Cd^{2+}	5.41	4.50	2.78			

EDTA



	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Mg^{2+} (T = 20 °C, μ = 0.1 M)	8.79					
Ca^{2+} (T = 20 °C, μ = 0.1 M)	10.69					
Ba^{2+} (T = 20 °C, μ = 0.1 M)	7.86					
Bi^{3+} (T = 20 °C, μ = 0.1 M)	27.8					
Co^{2+} (T = 20 °C, μ = 0.1 M)	16.31					
Ni^{2+} (T = 20 °C, μ = 0.1 M)	18.62					
Cu^{2+} (T = 20 °C, μ = 0.1 M)	18.80					

EDTA



	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Cr^{3+} (T = 20 °C, μ = 0.1 M)	(23.4)					
Fe^{3+} (T = 20 °C, μ = 0.1 M)	25.1					
Ag^{+} (T = 20 °C, μ = 0.1 M)	7.32					
Zn^{2+} (T = 20 °C, μ = 0.1 M)	16.50					
Cd^{2+} (T = 20 °C, μ = 0.1 M)	16.46					
Hg^{2+} (T = 20 °C, μ = 0.1 M)	21.7					
Pb^{2+} (T = 20 °C, μ = 0.1 M)	18.04					
Al^{3+} (T = 20 °C, μ = 0.1 M)	16.3					

Fluoride

F^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Al^{3+} (μ = 0.5 M)	6.11	5.01	3.88	3.0	1.4	0.4

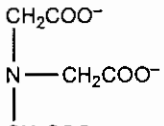
Hydroxide

OH^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Al^{3+}	9.01	(9.69)	(8.3)	6.0		
Co^{2+}	4.3	4.1	1.3	0.5		
Fe^{2+}	4.5	(2.9)	2.6	–0.4		
Fe^{3+}	11.81	10.5	12.1			
Ni^{2+}	4.1	3.9	3			
Pb^{2+}	6.3	4.6	3.0			
Zn^{2+}	5.0	(6.1)	2.5	(1.2)		

Iodide

I^-	log K_1	log K_2	log K_3	log K_4	log K_5	log K_6
Ag^{+} (T = 18 °C)	6.58	(5.12)	(1.4)			
Cd^{2+}	2.28	1.64	1.08	1.0		
Pb^{2+}	1.92	1.28	0.7	0.6		

Nitriloacetate



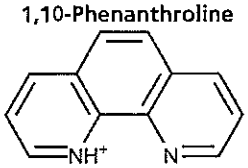
	log K_1	log K_2	log K_3	logK_4	log K_5	log K_6
Mg^{2+} (T = 20 °C, μ = 0.1 M)	5.41					
Ca^{2+} (T = 20 °C, μ = 0.1 M)	6.41					
Ba^{2+} (T = 20 °C, μ = 0.1 M)	4.82					
Mn^{2+} (T = 20 °C, μ = 0.1 M)	7.44					
Fe^{2+} (T = 20 °C, μ = 0.1 M)	8.33					
Co^{2+} (T = 20 °C, μ = 0.1 M)	10.38					
Ni^{2+} (T = 20 °C, μ = 0.1 M)	11.53					
Cu^{2+} (T = 20 °C, μ = 0.1 M)	12.96					
Fe^{3+} (T = 20 °C, μ = 0.1 M)	15.9					
Zn^{2+} (T = 20 °C, μ = 0.1 M)	10.67					
Cd^{2+} (T = 20 °C, μ = 0.1 M)	9.83					
Pb^{2+} (T = 20 °C, μ = 0.1 M)	11.39					

continued

Metal–Ligand Formation Constants—*continued*

Oxalate $C_2O_4^{2-}$	log K_1	log K_2	log K_3	logK_4	log K_5	log K_6
Ca^{2+} (μ = 1 M)	1.66	1.03				
Fe^{2+} (μ = 1 M)	3.05	2.10				
Co^{2+}	4.72	2.28				
Ni^{2+}	5.16					
Cu^{2+}	6.23	4.04				
Fe^{3+} (μ = 0.5 M)	7.53	6.11	4.85			
Zn^{2+}	4.87	2.78				

1,10-Phenanthroline



	log K_1	log K_2	log K_3	logK_4	log K_5	log K_6
Fe^{2+}			20.7 (β_3)			
Mn^{2+} (μ = 0.1 M)	4.0	3.3	3.0			
Co^{2+} (μ = 0.1 M)	7.08	6.64	6.08			
Ni^{2+} (μ = 0.1 M)	8.6	8.1	7.6			
Fe^{3+}			13.8 (β_3)			
Ag^{+} (μ = 0.1 M)	5.02	7.04				
Zn^{2+}	6.2	(5.9)	(5.2)			

Thiosulfate $S_2O_3^{2-}$	log K_1	log K_2	log K_3	logK_4	log K_5	log K_6
Ag^{+} (T = 20 °C)	8.82	4.85	0.53			

Thiocyanate SCN^{-}	log K_1	log K_2	log K_3	logK_4	log K_5	log K_6
Mn^{2+}	1.23					
Fe^{2+}	1.31					
Co^{2+}	1.72					
Ni^{2+}	1.76					
Cu^{2+}	2.33					
Fe^{3+}	3.02					
Ag^{+}	4.8	3.43	1.27	0.2		
Zn^{2+}	1.33	0.58	0.09	–0.4		
Cd^{2+}	1.89	0.89	0.02	–0.5		
Hg^{2+}		17.26 (β_2)	2.71	1.83		

Source: All values are from Martell, A. E.; Smith, R. M. *Critical Stability Constants,* Vol. 1–4, Plenum Press: New York, 1976. Unless otherwise stated, values are for 25 °C and zero ionic strength. Values in parentheses are considered less reliable.

Stability of Coordination Compounds

When the term stability is applied to coordination compounds (metal complexes) there can be two interpretations, **thermodynamic** or **kinetic** stability. Thermodynamic stability refers to the change in energy on going from reactants to products, i.e., ΔG for the reaction. Recall that $\Delta G=\Delta H-T\Delta S=-RT\ \ln K$, where ΔH is the enthalpy, ΔS the entropy and K is the equilibrium constant for the reaction. Kinetic stability refers to reactivity, generally ligand substitution. Substitution occurs extremely rapidly in some cases and extremely slowly in others. Complexes of the former type are referred to as labile and those of the latter type inert. Sometimes these two types of stability parallel one another, but often they do not, *vide infra*.

Thermodynamic stability often refers to the energetics and associated equilibrium constant for the reaction of an aquated metal ion with some other ligand (other than water).

$$[Cu(OH_2)_4]^{2+} + 4\ NH_3 \rightleftharpoons [Cu(NH_3)_4]^{2+} + 4\ H_2O$$

The overall equilibrium constant expression for this reaction, generally referred to as β_4 is defined in the usual fashion as $\beta_4 = \dfrac{[Cu(NH_3)_4]^{2+}}{[Cu^{2+}][NH_3]^4}$. Note that overall equilibrium constants are designated as β's and values for individual (step-wise) reactions as K's. For simplicity the water molecules associated with the Cu^{2+} have been deleted. This reaction can be described as four individual reactions with individual step-wise equilibrium constants K_1, K_2....K_4, i.e.,

$$[Cu(OH_2)_4]^{2+} + NH_3 \rightleftharpoons [Cu(OH_2)_3(NH_3)]^{2+} + H_2O \qquad \log K_1 = 4.22$$

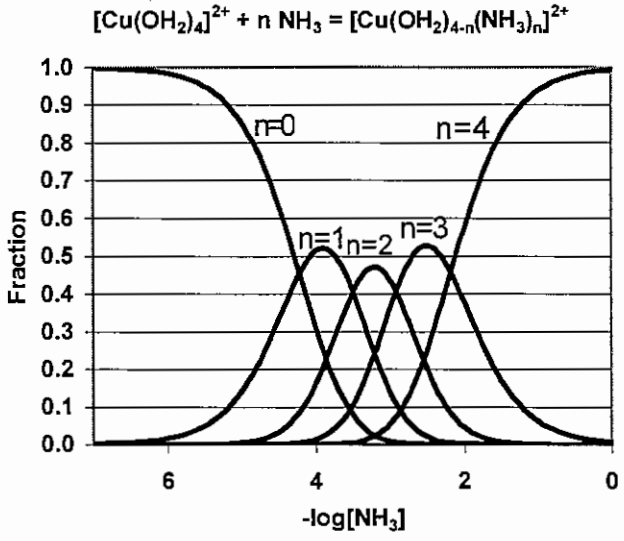
$$[Cu(OH_2)_3(NH_3)]^{2+} + NH_3 \rightleftharpoons [Cu(OH_2)_2(NH_3)_2]^{2+} + H_2O \qquad \log K_2 = 3.50$$

$$[Cu(OH_2)_2(NH_3)_2]^{2+} + NH_3 \rightleftharpoons [Cu(OH_2)(NH_3)_3]^{2+} + H_2O \qquad \log K_3 = 2.92$$

$$[Cu(OH_2)(NH_3)_3]^{2+} + NH_3 \rightleftharpoons [Cu(NH_3)_4]^{2+} + H_2O \qquad \log K_4 = 2.18$$

The decrease in successive step-wise constants is invariably observed and is due to several factors including statistics (number of replaceable positions), and increased steric interactions between the new ligands. Overall equilibrium β values from 1 to 4 can be defined as K_1, K_1K_2, $K_1K_2K_3$, and $K_1K_2K_3K_4$.

The speciation of copper(II) as a function of free ammonia concentration is shown in the Figure.



Equilibrium constants defined as shown above are termed formation constants or stability constants. Those for the reverse reaction are instability or dissociation constants. There can be some confusion here because in certain areas of chemistry and biology the term "stability constant" actually is defined as the dissociation constant.

The stability of the complex of a metal ion with a bidentate ligand such as ethylenediamine (en) is invariably significantly greater than the complex of the same ion with two monodentate ligands of comparable donor ability, i.e., for example two ammonia molecule. The is illustrated by the following data:

$$[Cu(OH_2)_4]^{2+} + en \rightleftharpoons [Cu(OH_2)_2(en)]^{2+} + 2\,H_2O \qquad \log K_1 = 10.6$$

$$\Delta H = -54 \text{ kJ mol}^{-1}, \Delta S = 23 \text{ J K}^{-1} \text{ mol}^{-1}$$

$$[Cu(OH_2)_4]^{2+} + 2\,NH_3 \rightleftharpoons [Cu(OH_2)_2(NH_3)_2]^{2+} + 2H_2O \qquad \log \beta_2 = 7.7$$

$$\Delta H = -46 \text{ kJ mol}^{-1}, \Delta S = -8.4 \text{ J K}^{-1} \text{ mol}^{-1}$$

This greater stability for complexes of chelate complexes is termed the **chelate effect.** Its origin is primarily in the differences in entropy between chelate and non-chelate complex reactions. The formation of chelate complexes results in greater disorder because of the formation of a larger number of free particles in the products whereas there is no change in the number of particles in the formation of comparable non-chelate complexes.

Ligand exchange is a good way to examine kinetic stability without complications from changes in ΔH for the reaction. Water exchange rates have been determined for a wide range of metal ions and oxidation states. A selection of these is given in the Table (next page). Although the absolute rate of exchange will differ for other ligands the values in the table can be used to gauge the relative reactivity of two metal ions or two different oxidation states of the same metal. There are several observations/conclusions that can be made from these data but before stating some of these some comments concerning the mechanism of these reactions are in order.

There are two limiting mechanisms for substitution reactions of coordination complexes, associative, which corresponds to the S_N2 reaction in organic chemistry, and dissociative, which corresponds to the S_N1 reaction in organic chemistry. Ligand exchange is a category of substitution reaction. In the limiting case of an associative reaction the entering ligand begins to interact with the metal as the bond to the departing ligand (leaving group) lengthens. In order for the entering ligand to bond to the metal there must be a vacant (preferably) or partially vacant orbital that is accessable to the entering ligand. In an octahedral complex only the t_{2g} d orbitals are accessible as the e_g orbitals and the n+1 s and p orbitals are blocked by the presence of the original six ligands. Associative processes are more likely for larger metal ions than for smaller so that they are more important early in a transition series and for heavier members of a family.

In the limiting case the dissociative process involves the formation of a five-coordinate intermediate as a result of complete bond dissociation to the departing ligand (leaving group). The five-coordinate intermediate then reacts with the new ligand (entering group) to reform a six-coordinate complex.

There are many subtleties to mechanisms of substitution of coordination complexes including simultaneous reaction by both pathways, but in the limit the associative mechanism is a second order reaction, first order in both complex and entering group (L), i.e., rate = k[complex][L]. Dissociative processes are first order in complex only since the entering group is not involved in the rate limiting step, which is bond cleavage between the metal ion and the leaving group. The rate law is then rate = k[complex].

Rate constants[a] for water exchange

$[ML_n(OH_2)]^{n+}$	k/s^{-1}	$[ML_n(OH_2)]^{n+}$	k/s^{-1}
		$[Ti(OH_2)_6]^{3+}$	1.8×10^{5}
$[V(OH_2)_6]^{2+}$	8.7×10^{1}	$[V(OH_2)_6]^{3+}$	5.0×10^{2}
$[Cr(OH_2)_6]^{2+}$	$>10^{8}$	$[Cr(OH_2)_6]^{3+}$	2.4×10^{-6}
$[Mn(OH_2)_6]^{2+}$	2.1×10^{7}		
$[Fe(OH_2)_6]^{2+}$	4.4×10^{6}	$[Fe(OH_2)_6]^{3+}$	1.2×10^{2}
$[Ru(OH_2)_6]^{2+}$	1.8×10^{-2}	$[Ru(OH_2)_6]^{3+}$	3.5×10^{-6}
$[Co(OH_2)_6]^{2+}$	3.2×10^{6}		
$[Ni(OH_2)_6]^{2+}$	3.2×10^{4}		
$[Pd(OH_2)_4]^{2+}$	5.6×10^{-2}		
$[Pt(OH_2)_4]^{2+}$	3.9×10^{-4}		
$[Cu(OH_2)_6]^{2+}$	$>10^{7}$		
$[Zn(OH_2)_6]^{2+}$	$>10^{7}$		
		$[Cr(NH_3)_5OH_2]^{3+}$	5.2×10^{-5}
		$[Co(NH_3)_5OH_2]^{3+}$	5.7×10^{-6}
		$[Rh(NH_3)_5OH_2]^{3+}$	8.4×10^{-6}
		$[Ir(NH_3)_5OH_2]^{3+}$	6.1×10^{-8}

[a]All rate constants are expressed as first order rate constants for comparative purposes even though some reactions are associative.

(1) An increase in oxidation state for the metal reduces the rate of exchange although there are obvious exceptions to this as indicated by the data for V(II) and V(III), and the exchange rate for Ti(III) appears very large compared to those of other trivalent ions (and even some divalent ions). The reason for this is that there is a large degree of associative character to reactions of the larger elements in a transition series and the activation energy for these reactions is directly related to the occupancy of the t_{2g} orbitals. Ti(III) has only one t_{2g} electron and V(III) only two, whereas V(II) has three t_{2g} electrons.

(2) The fact that water exchange occurs more rapidly in $[Rh(NH_3)_5OH_2]^{3+}$ than in the corresponding Co(III) complex is attributed to a change in mechanism for the larger rhodium complex. There are two reasons for this. First, the much smaller Co^{3+} ion (0.53 Å) can only react by a dissociative process, whereas the t_{2g} orbitals of the larger rhodium ion (0.67 Å, Ir^{3+} = 0.73 Å) are accessible to the entering group. Also bond strengths increase on going to heavier members of a family so that dissociative processes are necessarily slower.

(3) The much slower substitution of V(II) relative to the other first-row, divalent elements is also related to the absence of any e_g electrons. In the crystal field model the presence of e_g electrons means that there are increased electron-electron repulsions with ligand electron pairs and therefore longer and weaker bonds. In the molecular orbital model the e_g orbitals are antibonding with respect to the metal-ligand interaction and the presence of electrons in these orbitals reduces the bond order for the metal-ligand interaction.

This point is also dramatically illustrated for the Fe(II)/Ru(II) and Ni(II)/Pd(II) complexes. The Fe(II), which is high spin, has two e_g electrons, whereas the Ru(II) complex is low-spin and therefore has no e_g electrons. Spin pairing in the Ru(II) complex is a result of the increased strength of its interaction with the six ligands, which also contributes to its decreased reactivity. The Ni(II) complex also has two e_g electrons, whereas the four-coordinate, planar Pd(II) complex, like all planar d^8 complexes is diamagnetic. Clearly there are multiple effects here, but the absence of antibonding electrons and the greater metal-ligand bond strength are important contributors to the much lower reactivity of the Pd complex. Note that the reactivity of the Pt analog is still lower.

In the introduction it was indicated that thermodynamic stability and kinetic stability often parallel one another but that sometimes they do not. A vivid illustration of a case where they do not parallel one another is illustrated by the formation constant and ligand exchange rate constant for $[Ni(CN)_4]^{2-}$. The second order rate constant for CN^- exchange is $>5 \times 10^5\ M^{-1}\ s^{-1}$. However the complex is very stable since

$$\beta_4 = \frac{[Ni(CN)_4{}^{2-}]}{[Ni(OH_2)_6{}^{2+}][CN^-]^4} = 1.3 \times 10^{30}.$$

The high rate of ligand exchange is not so surprising when one considers that both axial

positions in the square-planar complex are accessible and that salts of the square-pyramidal complex $[Ni(CN)_5]^{3-}$ have been crystallized (and structurally characterized by x-ray diffraction).

WHO/SDE/WSH/05.08/55
English only

Nickel in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, and the first addendum to the third edition was published in 2005.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health

Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

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The work of the following working group coordinators was crucial in the development of this document and others contributing to the first addendum to the third edition:

Dr J. Cotruvo, J. Cotruvo Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Prof. Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
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The draft text was discussed at the Working Group Meeting for the first addendum to the third edition of the GDWQ, held on 17–21 May 2004. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinator was Dr J. Bartram, Coordinator, Water, Sanitation and Health Programme, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr Robert Bos, Water, Sanitation and Health Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

DNA	deoxyribonucleic acid
EDTA	edetic acid; ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for Drinking-water Quality*
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
TDI	tolerable daily intake
USA	United States of America
WHO	World Health Organization

Table of contents

1. GENERAL DESCRIPTION

1.1 Identity

Nickel is a lustrous white, hard, ferromagnetic metal. It occurs naturally in five isotopic forms: 58 (67.8%), 60 (26.2%), 61 (1.2%), 62 (3.7%), and 64 (1.2%).

1.2 Physicochemical properties

Property	*Value*
Specific density	8.90 g/cm^3 at 25 °C
Melting point	1555 °C
Boiling point	2837 °C

Nickel usually has two valence electrons, but oxidation states of +1, +3, or +4 may also exist. Metallic nickel is not affected by water but is slowly attacked by dilute hydrochloric or sulfuric acid and is readily attacked by nitric acid. Fused alkali hydroxides do not attack nickel. Several nickel salts, such as the acetate, chloride, nitrate, and sulfate, are soluble in water, whereas carbonates and hydroxides are far less soluble and sulfides, disulfides, subsulfides, and oxides are practically insoluble in water. Alloys of nickel containing more than 13% chromium are to a high degree protected from corrosion in many media by the presence of a surface film consisting mainly of chromium oxide (Morgan & Flint, 1989; Haudrechy et al., 1994).

1.3 Major uses and sources in drinking-water

The primary source of nickel in drinking-water is leaching from metals in contact with drinking-water, such as pipes and fittings. However, nickel may also be present in some groundwaters as a consequence of dissolution from nickel ore-bearing rocks.

Nickel is used principally in its metallic form combined with other metals and non-metals as alloys. Nickel alloys are characterized by their hardness, strength, and resistance to corrosion and heat.

Nickel is used mainly in the production of stainless steels, non-ferrous alloys, and super alloys. Other uses of nickel and nickel salts are in electroplating, as catalysts, in nickel–cadmium batteries, in coins, in welding products, and in certain pigments and electronic products (IARC, 1990). It is estimated that 8% of nickel is used for household appliances (IPCS, 1991). Nickel is also incorporated in some food supplements, which can contain several micrograms of nickel per tablet (EU, 2004).

1.4 Environmental fate

Nickel occurs predominantly as the ion $Ni(H_2O)_6^{2+}$ in natural waters at pH 5–9 (IPCS, 1991). Complexes with ligands, such as OH^-, SO_4^{2-}, HCO_3^-, Cl^-, and NH_3, are formed to a minor degree in this pH range.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

Nickel concentrations in remote areas are in the range of 1–3 ng/m^3, whereas concentrations in rural and urban air range from 5 to 35 ng/m^3. It has been estimated that non-occupational exposure via inhalation is 0.2–1.0 μg/day in urban areas and 0.1–0.4 μg/day in rural areas (Bennett, 1984). The mainstream smoke of one cigarette contains about 0.04–0.58 μg of nickel (IARC, 1990).

2.2 Water

Nickel concentrations in groundwater depend on the soil use, pH, and depth of sampling. The average concentration in groundwater in the Netherlands ranges from 7.9 μg/litre (urban areas) to 16.6 μg/litre (rural areas). Acid rain increases the mobility of nickel in the soil and thus might increase nickel concentrations in groundwater (IPCS, 1991). In groundwater with a pH below 6.2, nickel concentrations up to 980 μg/litre have been measured (RIVM, 1994).

In Canada, the median nickel level in drinking-water supplies was below the detection limit of 2 μg/litre; the maximum level observed was 69 μg/litre (Méranger et al., 1981). In drinking-water in the USA, 90% of all samples (n = 2503) contained ≤10 μg/litre, and 97% had nickel concentrations of ≤20 μg/litre (ATSDR, 1996).

In Europe, reported nickel concentrations in drinking-water were generally below 10 μg/litre (IPCS, 1991). Nickel levels below 1 μg/litre have been reported from Denmark and Finland (Punsar et al., 1975; Gammelgaard & Andersen, 1985). Average dissolved nickel concentrations in surface water in the rivers Rhine and Meuse are below 7 μg/litre (RIWA, 1994).

Increased nickel concentrations in groundwater and municipal tap water (100–2500 μg/litre) in polluted areas and areas in which natural nickel was mobilized have been reported (McNeely et al., 1972; Hopfer et al., 1989). Water left standing overnight in plumbing fittings plated with chromium on a base of nickel contained a nickel concentration of 490 μg/litre (Andersen et al., 1983).

Certain stainless steel well materials were identified as the source of increased nickel concentrations in groundwater wells in Arizona, USA. Mean nickel levels were 8–395 μg/litre; in some cases, nickel levels were in the range 1–5 mg/litre (Oakley & Korte, 1996).

Leaching of nickel from chromium–nickel stainless steel pipework into drinking-water diminished after a few weeks; as chromium was rarely found at any time in the water, this indicates that the leakage of nickel is not of corrosive origin, but rather attributable to passive leaching of nickel ions from the surface of the pipes (Schwenk, 1992). Concentrations of nickel leaching from new stainless steel pipes used for

drinking-water were up to 6 µg/litre (Nickel Development Institute, personal communication, 2004). This maximum concentration can be increased when the pipes are assembled with tinned copper and gunmetal fittings. Fittings such as taps, which are chromium-plated, release much higher concentrations, but these decrease significantly with time (EU, 2004).

Concentrations of nickel in water boiled in electric kettles may, depending on the material of the heating element, be markedly increased, especially in the case of new or newly decalcified kettles. The greatest concentrations are associated with nickel-plated elements; however, leaching decreases over time. Nickel concentrations in the range 100–400 µg/litre, with extreme values over 1000 µg/litre, have been reported (Rasmussén, 1983; Pedersén & Petersén, 1995; Berg et al., 2000; United Kingdom Drinking Water Inspectorate, 2002; EU, 2004).

Nickel concentrations in bottled mineral water will depend on the source and any treatment applied. Levels of nickel in a selection of bottled mineral waters were below the detection limit of 25 µg/litre (Allen et al., 1989).

2.3 Food

Since nickel is usually measured in food as total nickel, there is uncertainty as to the chemical form, although it is normally considered to be in the form of complex bound organic nickel, which may be less bioavailable than other forms (EU, 2004). Nickel levels in food are generally in the range 0.01–0.1 mg/kg, but there are large variations (Booth, 1990; Jorhem & Sundström, 1993; Dabeka & McKenzie, 1995; Fødevaredirektoratet, 2000). Higher median levels of nickel (0.1–0.4 mg/kg) were found in wholemeal products (Smart & Sherlock, 1987; Fødevaredirektoratet, 2000), whereas markedly higher levels (1–6 mg/kg) were found in beans, seeds, nuts, and wheat bran (Smart & Sherlock, 1987; Jorhem & Sundström, 1993). Even higher nickel levels (8–12 mg/kg) were found in cacao (Smart & Sherlock, 1987).

Stainless steel cooking utensils (e.g., oven pans, roasting pans) contributed markedly to the levels of nickel in cooked food, sometimes exceeding 1 mg/kg in meat (Dabeka & McKenzie, 1995), although there may be some questions regarding analytical contamination in this study. In contrast, Flint & Packirisamy (1995) found only minor increases in nickel concentrations in acid foodstuffs when new stainless steel pans were used.

Daily dietary intakes of nickel were 0.14–0.15 mg in the United Kingdom in 1981–1984 (Smart & Sherlock, 1987), 0.082 mg in Sweden in 1987 (Becker & Kumpulainen, 1991), 0.16 mg (mean; 95% fractile, 0.27 mg) in Denmark (Fødevaredirektoratet, 2000), and 0.16 mg in the USA (Myron et al., 1978). The dietary intake of nickel in a Canadian study ranged from 0.19 mg/day for 1- to 4-year-old children to 0.406 mg/day for 20- to 39-year-old males. The nickel intake for 20- to 39-year-old women was on average 0.275 mg/day (Dabeka & McKenzie, 1995). Dietary nickel intake by 0- to 12-month-old infants was on average 0.005 mg/kg of

body weight per day (equal to 0.038 mg/day). Infants fed evaporated milk were exposed to 0.004 mg/kg of body weight per day, whereas infants fed soy-based formula were exposed to 0.010 mg/kg of body weight per day (Dabeka, 1989). Nickel is found in both human and cow's milk at concentrations reported to range from 0.001 to over 0.1 mg/litre, although concentrations in studies in the USA indicate levels in the region of 0.015 mg/kg (EU, 2004). USFDA (2000) estimated an intake of 0.134 mg/day based on data from the northeastern part of the USA.

As nuts and beans are important sources of protein for vegetarians, this population group can be expected to have a markedly higher intake of nickel than that reported in the studies cited above. The nickel intake of eight volunteers ingesting normal diets averaged 0.13 mg/day (range 0.06–0.26 mg/day), compared with 0.07 mg/day (range 0.02–0.14 mg/day) when diets containing low nickel levels were consumed. When food rich in nickel was ingested, the daily intake was 0.25 mg/day (range 0.07–0.48 mg/day) (Veien & Andersen, 1986). A duplicate-diet study of vegetarians in the United Kingdom indicated an average dietary intake of nickel of 0.17 mg/day (FSA, 2000).

There is a great deal of concordance between the different studies of dietary intake, with the overall assessment that diet provides less than 0.2 mg/day.

2.4 Estimated total exposure and relative contribution of drinking-water

Food is the dominant source of nickel exposure in the non-smoking, non-occupationally exposed population. According to the 1981 United Kingdom Total Diet Study, the contribution from food is 0.22–0.23 mg/day per person. Later studies indicate that this is probably excessive, and recent studies, including a United Kingdom study on vegetarians, indicate that the intake from food is probably less than 0.2 mg/day. Water generally contributes 0.005–0.025 mg daily (i.e., 2–11% of the total daily oral intake of nickel) (MAFF, 1985). These figures are similar to those presented in the European risk assessment for nickel (EU, 2004). However, no account is taken of exposure from nickel-plated elements and other similar sources; for some individuals, therefore, there may be higher intakes that will fluctuate significantly with time. Overall, drinking-water appears to contribute only a minor proportion of daily intake, although exposure of some communities may be significant in specific circumstances where nickel levels in groundwater are unusually high.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

3.1 Laboratory animals

Nickel is poorly absorbed from diets and is eliminated mainly in the faeces. Absorbed nickel is rapidly cleared from serum and excreted in urine (IPCS, 1991).

The mechanism for intestinal absorption of nickel is not clear. Iron deficiency increased intestinal nickel absorption *in vitro* and *in vivo*, indicating that nickel is partially absorbed by the active transfer system for iron absorption in the intestinal mucosal cells (Tallkvist et al., 1994). In perfused rat jejunum, saturation of nickel uptake was observed at high concentrations of nickel chloride (Foulkes & McMullen, 1986). Iron concentrations in rat tissues were increased by dietary nickel exposure (Whanger, 1973). Nickel is bound to a histidine complex, albumin, and alpha-2-macroglobulin in serum (Sarkar, 1984).

Absorption of soluble nickel compounds from drinking-water is higher than that from food. After 24 h, 10–34% of a single oral dose of water-soluble nickel compounds (i.e., $NiSO_4$, $NiCl_2$, $Ni(NO_3)_2$) was absorbed, whereas less than 2% of a single oral dose of insoluble or scarcely soluble nickel compounds (i.e., NiO, Ni, Ni_3S_2, NiS) was absorbed. It is not known if the animals were fasted before treatment. The highest nickel concentrations were found in the kidneys and lungs, whereas nickel concentrations in the liver were low (Ishimatsu et al., 1995).

Whole-body retention in mice after oral exposure to Ni^{2+} was less than 1% of the administered dose 5 days after exposure (Nielsen et al., 1993). Severa et al. (1995) observed an accumulation of nickel in organs of rats orally exposed to nickel in drinking-water at concentrations of 100 mg/litre for 6 months. The nickel concentration in liver was 10 times higher in exposed rats than in unexposed rats; in the kidney, the nickel level was only twice as high in exposed rats as in unexposed rats. Nickel levels in the kidney and blood were similar. There was no increase in nickel levels in organs between 3 and 6 months of exposure. Biliary excretion of nickel subcutaneously administered to rats as nickel chloride was less than 0.5% of the given dose (Marzouk & Sunderman, 1985).

Several reports indicate that transplacental transfer of nickel occurs in animals (IPCS, 1991). Elevated concentrations of nickel were detected in fetuses after intramuscular administration of nickel chloride to rats. The fetal organ with the highest nickel concentration was the urinary bladder (Sunderman et al., 1978).

A dose-dependent increase in nickel concentrations in rat milk was observed after a single subcutaneous injection of nickel chloride. The milk/plasma ratio was 0.02 (Dostal et al., 1989).

3.2 Humans

Following a 12-h fast, a volunteer ingested 20 µg of ^{61}Ni-enriched nickel per kg of body weight as nickel nitrate in 1 litre of water. The serum nickel concentration peaked at 2 h at 34 µg/litre. By 96 h, 27% of the ingested dose was excreted in the urine (Templeton et al., 1994a). These findings are consistent with the observations made by Sunderman and co-workers, who reported an absorption of 27 ± 17% of the given nickel dose (as nickel sulfate) added to drinking-water in 10 volunteers after a 12-h fast. Intestinal absorption was only 1% of the given dose when nickel as sulfate

salt was added to scrambled eggs. The half-time for absorbed nickel averaged 28 ± 9 h (Sunderman et al., 1989). Plasma levels in fasting human subjects did not increase above fasting levels when 5 mg of nickel were added to an American breakfast or a Guatemalan meal rich in phytic acids (Solomons et al., 1982). The same amount of nickel added to water elevated the plasma nickel levels 4- to 7-fold. The absorption of nickel added to milk, tea, coffee, or orange juice was significantly less than the absorption of nickel from water. Two studies carried out to examine the influence of fasting and food intake on the absorption of nickel from drinking-water showed that a dose of 12 µg/kg of body weight given to fasted males in drinking-water was more rapidly absorbed if the dose was given 30 min or 1 h before a meal of scrambled eggs than if given at the same time. The peak concentration in blood was also 13-fold higher. In a similar experiment in which ^{61}Ni was given to 20 nickel-sensitized women and 20 age-matched controls, there was no difference in nickel absorption and excretion (Nielsen et al., 1999).

A fatal case of nickel intoxication indicates that biliary excretion of nickel is of minor importance in humans (Grandjean et al., 1989).

According to the above studies, the daily amount of absorbed nickel in humans will be, on average, about 10 µg from food and about 5 µg or less from water.

Nickel has been detected in fetal tissues at levels similar to the levels found in adults (McNeely et al., 1972; Casey & Robinson, 1978).

Serum levels in the range 1.5–19 µg/litre were found in patients undergoing regular haemodialysis (Hopfer et al., 1989; Nixon et al., 1989). Significantly higher serum nickel levels were observed in non-occupationally exposed subjects from a heavily nickel-polluted area compared with levels in subjects living in a control area (nickel concentrations in tap water 109 ± 46 vs 0.6 ± 0.2 µg/litre; serum nickel levels 0.6 ± 0.3 vs 0.2 ± 0.2 µg/litre) (Hopfer et al., 1989). Tentative reference values for nickel in serum and urine have been proposed: 0.2 µg/litre or lower in serum, and 1–3 µg/litre in urine of healthy adults (Templeton et al., 1994b).

Nickel is also eliminated in the milk of lactating women. In studies reported in the USA, the nickel concentration in milk was in the region of 15 µg/kg (EU, 2004).

4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO *SYSTEMS*

4.1 Acute exposure

Effects on kidney function, including tubular and glomerular lesions, have been reported by several authors after parenteral administration of high nickel doses of between 1 and 6 mg/kg of body weight intraperitoneally in rabbits and rats (IPCS, 1991).

4.2 Short-term exposure

Body weight gain, haemoglobin, and plasma alkaline phosphatase were significantly reduced in weanling rats exposed to nickel (as nickel acetate) at concentrations of 500 or 1000 mg/kg in the diet (equivalent to 25 or 50 mg/kg of body weight per day) for 6 weeks compared with controls (Whanger, 1973). No effects were observed in rats exposed to 100 mg/kg in the diet (equivalent to 5 mg/kg of body weight per day).

In a 13-week study in which Sprague-Dawley rats were given 0, 44.7, 111.75, or 223.5 mg of nickel per litre in drinking-water as nickel sulfate (corresponding to 0, 4.5, 11.2, and 22.4 mg of nickel per kg of body weight per day), no apparent clinical signs of toxicity were observed. Final mean body weights were unaffected except for a decrease in the top dose group when compared with controls. Lymphocyte subpopulations (T and B cells) were induced at the lower doses but suppressed at the highest dose. No gross or microscopic changes were seen in any of the tissues examined (Obone et al., 1999). The EU (2004) risk assessment determined a NOAEL of 44.7 mg/litre based on minor changes in body weight and relative weights of kidney and lung.

4.3 Long-term exposure

Rats (25 per sex per dose) were exposed to nickel (as nickel sulfate) in the diet at doses of 0, 100, 1000, or 2500 mg/kg (equivalent to 0, 5, 50, and 125 mg/kg of body weight per day) for 2 years (Ambrose et al., 1976). Growth was depressed in rats at 1000 and 2500 mg/kg of diet, but there were indications that decreased food consumption might explain the decreased body weight gains, particularly at 2500 mg/kg of diet. However, no statistical analysis seems to have been performed. Survival was overall very poor, especially in the control groups and the 2500 mg/kg of diet groups. In females at 1000 and 2500 mg/kg of diet, the mean relative liver weights were decreased by about 20% and the mean relative heart weights were increased by about 30% compared with the control group. No histological or gross pathological findings related to nickel exposure were observed. The highest nickel concentrations were found in the kidneys. The NOAEL in this study was 5 mg/kg of body weight per day. However, the study does not meet current standards for long-term studies, mainly because of the low survival rate. The observed changes in organ weights in female rats might in part be due to changes in food and water consumption. Also, both gross and histopathological examinations of the animals were negative, although there were 20–30% changes in relative organ weights. It can thus not be excluded that the observed changes in relative organ weights were related to changes in food and/or water consumption rather than to a toxic effect of nickel.

Increased relative kidney weight was observed in rats exposed to nickel (as nickel sulfate) in drinking-water at a daily dose of about 7 mg/kg of body weight for up to 6 months (Vyskocil et al., 1994). There was an increased excretion of albumin in urine in females, but there were no changes in total protein, beta-2-microglobulin, *N*-acetyl-beta-D-glucosaminidase, or lactate dehydrogenase in urine due to nickel exposure.

In a 2-year study, dogs (three per sex per dose) were exposed to 0, 100, 1000, or 2500 mg of nickel per kg of diet (equivalent to 0, 2.5, 25, and 62.5 mg/kg of body weight per day). In the 2500 mg/kg of diet group, decreased weight gain and food consumption, higher kidney to body weight and liver to body weight ratios, and histological changes in the lung were observed. The NOAEL in this study was 25 mg/kg of body weight per day (Ambrose et al., 1976).

4.4 Reproductive and developmental toxicity

Intraperitoneal administration of nickel nitrate (12 mg of nickel per kg of body weight) to male mice resulted in reduced fertilizing capacity of spermatozoa; no effects were seen at 8 mg of nickel per kg of body weight (Jacquet & Mayence, 1982).

A reduced number of live pups and reduced body weights of fetuses were observed in rats exposed to single doses of nickel chloride (16 mg of nickel per kg of body weight) or nickel subsulfide (80 mg of nickel per kg of body weight) administered intramuscularly on day 8 and day 6, respectively. No congenital anomalies were found in the fetuses (Sunderman et al., 1978).

Velazquez & Poirer (1994) and ATSDR (1996) described a two-generation study in rats. Nickel chloride was administered in drinking-water at concentrations of 0, 50, 250, or 500 mg/litre (equal to 0, 7, 31, and 52 mg of nickel per kg of body weight per day) from 90 days before breeding. Along with changes in maternal body weight and liver weight at the 500 mg/litre dose level in the P_0 generation, there were also a dose-related decrease in live litter size and pup weight and increased neonatal mortality. In the F_1 generation, there was dose-related mortality between 3 and 7 weeks of age at the 250 and 500 mg/litre dose levels. For the F_1 matings, there were also dose-related decreases in live litter size and increased mortality per litter, but this was significant only in the high-dose group. Decreased food intake and water intake were observed in the exposed animals. Also, the room temperature was up to 6 °C higher than normal at certain times during gestation and the early postnatal days. Lower than normal levels of humidity were also recorded. Thus, the NOAEL in this study is considered to be 7 mg of nickel per kg of body weight per day; however, because of the problems referred to, it is difficult to make a direct association between the effects reported in this study and nickel exposure.

Female Long-Evans rats were exposed to nickel as nickel chloride for 11 weeks prior to mating and then during two successive gestation periods (G1 and G2) and lactation periods (L1 and L2) at concentrations of 0, 10, 50, or 250 mg/litre (equal to 0, 1.3, 6.8, and 31.6 mg of nickel per kg of body weight per day) in drinking-water (Smith et al., 1993). Dams drinking water containing nickel at 31.6 mg/kg of body weight per day consumed less liquid and more food per kg of body weight than did controls. Maternal weight gain was reduced during G1 in the mid- and high-dose groups. There were no effects on pup birth weight, and weight gain was reduced only in male pups from dams in the mid-dose group. The proportion of dead pups per litter was

significantly elevated at the high dose in L1 and at the low and high doses in L2 (the increase at the middle dose in L2 approached statistical significance), with a dose-related response in both experimental segments. The number of dead pups per litter was significantly increased at each dose in L2. It was noted that the number of litters with dead pups and the total number of dead pups per litter in the control group were less in L2 than in L1. Plasma prolactin levels were reduced in dams at the highest dose level 1 week after weaning of the second litter. The authors concluded that 1.3 mg/kg of body weight per day represented the LOAEL in this study, although this is considered to be conservative, owing to variations in response between the successive litters.

A range-finding study was carried out for a two-generation study investigating the potential for reproductive toxicity of nickel (SLI, 2000; EU, 2004). The range-finding and definitive studies for the rat two-generation reproduction study of nickel sulfate hexahydrate were conducted using gavage as the route of exposure, due to palatability problems with nickel in drinking-water and bioavailability problems with nickel in food. The range-finding study was designed in two parts. The first part was a dose–response probe utilizing small numbers of animals and nickel sulfate hexahydrate exposures of 0, 5, 15, 25, 50, 75, and 150 mg/kg of body weight per day. (Note that the lower 95% confidence limit for lethality from nickel sulfate hexahydrate is 170 mg/kg of body weight per day.) Lethality was observed at the 150 mg/kg of body weight per day exposure level.

The second part of the range-finding study (i.e., a one-generation reproductive toxicity study) utilized nickel sulfate hexahydrate exposures of 0, 10, 20, 30, 50, and 75 mg/kg of body weight per day. These doses had no effect on parental survival, growth, mating behaviour, copulation, fertility, implantation, or gestation length. However, evaluation of post-implantation/perinatal lethality among the offspring of the treated parental rats (i.e., the number of pups conceived minus the number of live pups at birth) showed statistically significant increases at the 30–75 mg/kg of body weight per day exposures and more questionable increases at the 10 and 20 mg/kg of body weight per day levels. The decrease in perinatal survival evident in the one-generation range-finding study was anticipated from previous literature reports. The goal of the range-finding studies was to refine the NOAEL for this end-point. The one-generation study also showed that the mean live litter size was significantly decreased at the 75 mg/kg of body weight per day level and was lower than historical controls at or above 30 mg/kg of body weight per day.

Based upon the results of the one-generation study, nickel sulfate hexahydrate exposure levels of 1, 2.5, 5.0, and 10 mg/kg of body weight per day were administered by gavage to five groups of male and female rats in the definitive two-generation study. These dose levels were chosen to ensure that the study would have a measurable NOAEL for the post-implantation/perinatal lethality variable. Males of the parental (F_0) generation were dosed during growth and for at least one complete spermatogenic cycle in order to elicit any possible adverse effects on spermatogenesis by the test substance. Females of the F_0 generation were dosed during growth and for

several complete estrous cycles in order to elicit any possible adverse effects on estrus by the test substance. The test substance was administered to F_0 animals during mating, during pregnancy, and through the weaning of their first-generation (F_1) offspring. At weaning, the administration of the substance was continued to F_1 offspring during their growth into adulthood, mating, and production of an F_2 generation and up until the F_2 generation was weaned. Clinical observation and pathological examination were performed for signs of toxicity, with special emphasis on effects on the integrity and performance of the male and female reproductive systems and on the growth and development of the offspring. The results from the two-generation study indicate that the highest dose selected (10 mg/kg of body weight per day, or 2.2 mg of nickel per kg of body weight per day) was the NOAEL for adult and offspring rats for all the end-points studied, including the variable of post-implantation/perinatal lethality (SLI, 2000; EU, 2004).

In a three-generation study in rats at dietary levels of 250, 500, or 1000 mg of nickel (administered as nickel sulfate) per kg of diet (equivalent to 12.5, 25, or 50 mg/kg of body weight per day), a higher incidence of stillborns in the first generation was observed compared with the control group (Ambrose et al., 1976). Body weights were decreased in weanlings at 1000 mg/kg of diet in all generations. The number of pups born alive per litter and the number of pups weaned per litter were progressively fewer with increasing nickel dose, but no statistical analysis of the results is presented. Decreased weanling body weight is a clear-cut effect in the 1000 mg/kg of diet dose group. No teratogenic effects were observed in any generation at any dose level. No histological lesions were observed in the third generation at weaning.

Decreased litter sizes were observed in a small-scale three-generation study in rats administered nickel in drinking-water at 5 mg/litre, corresponding to 0.2 mg/kg of body weight per day (Schroeder & Mitchener, 1971).

Alterations in milk composition were observed in lactating rats exposed to four daily subcutaneous injections of nickel at doses of 3–6 mg/kg of body weight (Dostal et al., 1989). Liver weights were decreased in pups whose dams received 6 mg of nickel per kg of body weight. These findings may explain the effects seen on litter size and body weights of the pups in studies described above.

4.5 Mutagenicity and related end-points

Nickel compounds are generally inactive in bacterial mutation assays but active in mammalian cell systems (IPCS, 1991). It was concluded that nickel-induced responses involved cell toxicity in all gene mutation studies using mammalian cells.

Chromosomal gaps, deletions and rearrangements, DNA–protein cross-links, and sister chromatid exchanges are reported in mammalian systems, including human cell systems. Chromosomal aberrations occur in all chromosomes but with preference for the heterochromatic centromeric regions (IPCS, 1991; Rossman, 1994).

In several experimental systems, nickel ions have been shown to potentiate the effects of other mutagenic agents, which may be explained by the capacity of nickel to inhibit DNA repair (Lynn et al., 1994; Rossman, 1994).

The genotoxicity of nickel compounds has been reviewed by TERA (1999) and as part of the EU (2004) risk assessment. Most studies relate to water-soluble compounds, and the TERA (1999) review concluded that "evidence for genotoxicity is mixed, although water soluble nickel compounds have been generally consistent in inducing effects in certain kinds of mammalian assays, particularly mutagenic responses and DNA damage *in vitro*, chromosomal effects including aberrations and sister-chromatid exchanges *in vitro* and *in vivo*, and carcinogenic transformation of mammalian cells *in vitro*. Responses in many of these assays were weak and occurred at toxic doses."

4.6 Carcinogenicity

A number of studies on the carcinogenicity of nickel compounds in experimental animals are available (IARC, 1990; Aitio, 1995). Generally, tumours are induced at the site of administration of the nickel compound. For instance, several nickel compounds induce injection-site sarcomas (Sunderman, 1984). A marked variation in the incidence of injection-site sarcomas between different strains of mice has been reported (Rodriguez et al., 1996).

There are only a limited number of studies on carcinogenic effects after oral exposure to nickel compounds. The incidence of tumours was not higher in rats exposed to drinking-water containing nickel at 5 mg/litre during their lifetime compared with control rats (Schroeder et al., 1974). As well, no difference in tumour incidence was observed in a lifetime study in rats exposed to 5, 50, or 125 mg of nickel per kg of body weight per day in the feed compared with controls (Ambrose et al., 1976). Owing to the high death rate and lack of information on cause of death, this study is of minor value in evaluating carcinogenicity after oral exposure to nickel. A similar 2-year study in dogs also revealed no increase in tumours (Ambrose et al., 1976).

4.7 Other effects

Nickel salts affect the T-cell system and suppress the activity of natural killer cells in rats and mice (IPCS, 1991). Mitogen-dependent lymphocyte stimulation was inhibited in human lymphocytes (Sikora & Zeromski, 1995) and in spleens of mice exposed to nickel (IPCS, 1991). Dose-related decreased spleen proliferative response to lipopolysaccharide was observed in mice exposed to nickel sulfate in drinking-water for 180 days. At the lowest dose (44 mg of nickel per kg of body weight per day), decreased thymus weight was observed, but there was no nickel-induced immunosuppression NK cell activity or response to T-cell mitogens.

Parenteral administration of nickel to rabbits, chickens, and rats and oral administration of nickel to rabbits induce hyperglycaemia and reduce the levels of prolactin releasing factor in rats (IPCS, 1991).

The myeloid system was affected (i.e., decrease in bone marrow cellularity and dose-related reductions in the bone marrow proliferative response) when mice were exposed to nickel sulfate in drinking-water at doses of 0, 44, 108, or 150 mg of nickel per kg of body weight per day for 180 days (Dieter et al., 1988). The LOAEL in this study was 44 mg of nickel per kg of body weight per day.

5. EFFECTS ON HUMANS

5.1 Acute exposure

A 2½-year-old girl died after ingesting about 15 g of nickel sulfate crystals. Cardiac arrest occurred after 4 h; the autopsy revealed acute haemorrhagic gastritis (Daldrup et al., 1983).

Thirty-two industrial workers accidentally drank water contaminated with nickel sulfate and nickel chloride (1.63 g of nickel per litre). The nickel doses in persons who developed symptoms were estimated to range from 7 to 35 mg/kg of body weight. Twenty workers developed symptoms, including nausea, vomiting, diarrhoea, giddiness, lassitude, headache, and shortness of breath. In most cases, these symptoms lasted for a few hours, but they persisted for 1–2 days in seven cases. Transiently elevated levels of urine albumin suggesting mild transient nephrotoxicity were found in two workers 2–5 days after exposure. Mild hyperbilirubinaemia developed on day 3 after exposure in two subjects, and elevated levels of blood reticulocytes were observed in seven workers on day 8 post-exposure. It is known from animal studies that nickel after intrarenal injection enhances the renal production of erythropoietin, which may explain the reticulocytosis, and that nickel induces microsomal haem oxygenase activity in liver and kidney, leading to a secondary hyperbilirubinaemia. Serum nickel concentrations ranged between 13 and 1340 µg/litre in persons with symptoms (Sunderman et al., 1988).

Seven hours after ingesting nickel sulfate in drinking-water (50 µg of nickel per kg of body weight), a 55-year-old man developed left homonymous haemianopsia, which lasted 2 h (Sunderman et al., 1989).

Nickel intoxication in 23 patients receiving haemodialysis was reported (Webster et al., 1980). The dialysate was contaminated by leachate from a nickel-plated stainless steel water heater tank. Symptoms such as nausea, vomiting, headache, and weakness occurred rapidly after exposure at plasma nickel concentrations of about 3 mg/litre and persisted for 3–13 h after dialysis.

5.2 Skin irritation and hypersensitivity

Allergic contact dermatitis is the most prevalent effect of nickel in the general population. A recent epidemiological investigation showed that 20% of young (15–34 years) Danish women and 10% of older (35–69 years) women were nickel-sensitized, compared with only 2–4% of Danish men (15–69 years) (Nielsen & Menné, 1992). The prevalence of nickel allergy was found to be 7–10% in previously published reports (Menné et al., 1989). EDTA reduced the number and severity of patch test reactions to nickel sulfate in nickel-sensitive subjects (Allenby & Goodwin, 1983).

Systemically induced flares of dermatitis are reported after oral challenge of nickel-sensitive women with 0.5–5.6 mg of nickel as nickel sulfate administered in a lactose capsule (Veien, 1989). At the highest nickel dose (5.6 mg), there was a positive reaction in a majority of the subjects; at 0.5 mg, only a few persons responded with flares. Responses to oral doses of 0.4 or 2.5 mg of nickel did not exceed responses in subjects given placebos in double-blind studies (Jordan & King, 1979; Gawkrodger et al., 1986).

After an oral dose of 1 mg of nickel, significantly higher levels of nickel were found in the urine of atopic patients (i.e., persons with a history of flexural dermatitis) compared with controls, indicating a higher gastrointestinal absorption of nickel in atopic persons (Hindsén et al., 1994). No such difference was found between nickel-allergic patients and controls. The small number of patients may explain these unexpected findings.

There are several reports on the effects of diets low or high in nickel, but it is still a matter of discussion whether naturally occurring nickel in food may worsen or maintain the hand eczema of nickel-sensitive patients, mainly because results from dietary depletion studies have been inconclusive (Veien & Menné, 1990). In a single-blind study, 12 nickel-sensitive women were challenged with a supplementary high-nickel diet (Nielsen et al., 1990). The authors concluded that hand eczema was aggravated during the period (i.e., days 0–11) and that the symptoms thus were nickel-induced. However, it should be noted that in some subjects the severity of the eczema (i.e., the number of vesicles in the palm of the hand) varied markedly between day 14 or 21 before the challenge period and the start of the challenge period.

Oral hyposensitization to nickel was reported after six weekly doses of 5 mg of nickel in a capsule (Sjöwall et al., 1978) and 0.1 ng of nickel sulfate daily for 3 years (Panzani et al., 1995). Cutaneous lesions were improved in eight patients with contact allergy to nickel after oral exposure to 5 mg of nickel weekly for 8 weeks (Bagot et al., 1995). Nickel in water (as nickel sulfate) was given to 25 nickel-sensitive women in daily doses of 0.01–0.04 mg/kg of body weight per day for 3 months after they had been challenged once with 2.24 mg of nickel (Santucci et al., 1988). In 18 women, flares occurred after the challenge dose, whereas only 3 out of 17 subjects had symptoms during the prolonged exposure period. Later, Santucci and co-workers (1994) gave increasing oral doses of nickel in water (0.01–0.03 mg of nickel per kg of

body weight per day) to eight nickel-sensitive women for up to 178 days. A significant improvement in hand eczema was observed in all subjects after 1 month.

The LOAEL established after oral provocation of patients with empty stomachs was reported as 12 µg/kg of body weight (Nielsen et al., 1999). This figure was similar to the dose found in a study by Hindsén et al. (2001), where a total dose of 1 mg (17 µg/kg of body weight) was reported to result in a flare-up of dermatitis in an earlier patch test site in 2 of 10 nickel-sensitive patients. The dose of 12 µg/kg of body weight was considered to be the acute LOAEL in fasting patients on a 48-h diet with reduced nickel content. A cumulative LOAEL could be lower, but a LOAEL in non-fasting patients is probably higher because of reduced absorption of nickel ions when mixed in food.

5.3 Carcinogenicity

The identification of nickel species hazardous to humans was investigated by the International Committee on Nickel Carcinogenesis in Man by analysing 10 previously studied cohorts of men occupationally exposed to nickel (ICNCM, 1990). It was concluded that occupational exposure to sulfidic and oxidic nickel at high concentrations causes lung and nasal cancers. There was no correlation between metallic nickel exposure and cancer in lung or nose. Soluble nickel exposure increased the cancer risk and may also enhance the risk associated with exposure to less soluble nickel compounds. The Committee also concluded that there was no substantial evidence that nickel compounds may produce cancers other than in the lung or nose in occupationally exposed persons.

Inhalation is an important route of exposure to nickel and its salts in relation to health risks. IARC (1990) concluded that nickel compounds are carcinogenic to humans (Group 1), whereas metallic nickel is possibly carcinogenic to humans (Group 2B). However, there is a lack of evidence of a carcinogenic risk from oral exposure to nickel.

A number of subsequent epidemiological studies have also supported these earlier findings (TERA, 1999; EU, 2004), but there remain no data on oral exposure.

6. PRACTICAL ASPECTS

6.1 Analytical methods and analytical achievability

The two most commonly used analytical methods for nickel in water are atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry. Flame atomic absorption spectrometry is suitable in the range of 0.5–100 µg/litre (ISO, 1986), whereas inductively coupled plasma atomic emission spectroscopy can be used for the determination of nickel with a limit of detection of about 10 µg/litre (ISO, 1996). A limit of detection of 0.1 µg/litre or better should be achievable using inductively coupled plasma mass spectrometry. The limit of

detection is approximately 20 µg/litre by flame atomic absorption spectrometry, 15 µg/litre by inductively coupled plasma, 1 µg/litre by electrothermal atomic absorption spectrometry, and 1 µg/litre by inductively coupled plasma optical emission spectrometry. Alternatively, electrothermal atomic absorption spectrometry can be used.

6.2 Treatment and control methods and technical achievability

Nickel can be found in drinking-water as a consequence of its presence in alloys used in contact with drinking-water, chromium or nickel plating of fittings, or its presence in water sources, usually as a consequence of dissolution from naturally occurring nickel-bearing strata in groundwater. In the first two cases, control is by appropriate control of materials in contact with drinking-water or, in the second instance, education of consumers to flush chromium- or nickel-plated taps before using the water.

Conventional surface water treatment, comprising chemical coagulation, sedimentation, and filtration, can achieve 35–80% removal of nickel (Zemansky, 1974; Hunter et al., 1987; Duguet & Rizet, 1996). Better nickel removal occurs with waters containing high concentrations of suspended solids; for waters low in solids, the addition of powdered activated carbon can be used to enhance nickel removal (Welté, 2002). In a review of nickel removal, it was concluded that conventional coagulation, clarification, and granular activated carbon filtration can give nickel removals of 35–80%, depending on the speciation of the nickel. Increasing pH and the presence of high turbidity both favour nickel removal. The optimum pH for removal on activated carbon was reported to be pH 8 (Duguet & Rizet, 1996). However, other studies have reported that nickel is rather poorly adsorbed on activated carbon (Seco et al., 1997).

In the case of groundwaters, effective removal of nickel can be achieved using chelating ion-exchange resins (Stetter et al., 2002). Various adsorbents could potentially be used to remove nickel from groundwaters (Duguet & Rizet, 1996; Welté, 2002).

7. GUIDELINE VALUE

In a well conducted two-generation study on rats, a NOAEL of 2.2 mg of nickel per kg of body weight per day was identified for all the end-points studied, including the variable of post-implantation/perinatal lethality (SLI, 2000; EU, 2004). The application of an uncertainty factor of 100 (10 to account for interspecies variation and 10 to account for intraspecies variation) gives a TDI of 22 µg/kg of body weight. A general toxicity value of 130 µg/litre (rounded value) could be determined from this TDI by assuming a 60-kg adult drinking 2 litres of water per day and allocating a conservative 20% of the TDI to drinking-water, as data show that the exposure from food for the general population is moderate and that a higher exposure could be allowed from drinking-water. It should be noted that this general toxicity value is

higher than the previous provisional guideline value for nickel, as it is based on a better reproductive study with less uncertainty.

However, this general toxicity value may not be sufficiently protective of individuals sensitized to nickel, for whom a sufficiently high oral challenge has been shown to elicit an eczematous reaction. The guideline value for nickel in drinking-water is therefore derived using the LOAEL of 12 µg/kg of body weight established after provocation of fasted patients with an empty stomach (Nielsen et al., 1999). In this study, nickel was administered as a single dose at a level that is much higher than would normally be possible through drinking-water and/or with the presence of food in the stomach, which would significantly reduce the absorption. Because this LOAEL of 12 µg/kg of body weight is based on a highly sensitive human population, it is not necessary to include an uncertainty factor to derive the TDI. Assuming a 60-kg adult drinking 2 litres of water per day and allocating 20% of total daily intake to drinking-water, the guideline value is 70 µg/litre (rounded value), which would be considered protective of nickel-sensitive individuals, the group at risk. Although this is close to the acute LOAEL, the LOAEL is based on the total exposure to nickel, in this study, being from drinking-water, and the absorption of nickel from drinking-water on an empty stomach is 10- to 40-fold higher than the absorption from food. Basing the total acceptable intake for oral challenge from studies using drinking-water on an empty stomach in fasted patients can, therefore, be considered a worst-case scenario.

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Public Health England

Nickel

Toxicological Overview

Key Points

Kinetics and metabolism

- Nickel is absorbed by inhalation and ingestion
- The absorption of nickel is related to the solubility of the nickel compound
- Absorbed nickel is primarily excreted via the urine

Health effects of acute exposure

- Nickel carbonyl is the most toxic nickel compound following acute inhalation exposure
- The immediate effects of nickel carbonyl exposure are respiratory tract irritation and neurological effects
- Delayed effects of nickel carbonyl exposure include pulmonary oedema, pneumonitis and in severe cases death
- Acute ingestion of nickel compounds can cause nausea, vomiting, diarrhoea and headache
- Dermal contact with nickel or nickel compounds can lead to sensitisation and the development of contact dermatitis

Health effects of chronic exposure

- Chronic inhalation of nickel or nickel compounds can cause rhinitis, sinusitis, anosmia and in extreme cases perforation of the nasal septum
- The International Agency for Research on Cancer (IARC) classified nickel compounds as carcinogenic to humans (Group 1)
- The IARC classified elemental nickel as possibly carcinogenic to humans (Group 2B)
- Nickel carbonyl and soluble nickel salts are considered to be reproductive toxicants

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Toxicological Overview

Summary of Health Effects

Nickel carbonyl is the most toxic nickel compound following acute inhalation exposure in humans. The effects of nickel carbonyl inhalation occur in two phases, immediate and delayed. The immediate effects include respiratory tract irritation and neurological effects such as dizziness and headache, following which there is often an asymptomatic period before the onset of the delayed pulmonary symptoms, including chest pain, cough and dyspnoea. In severe cases pulmonary oedema, pneumonitis and death may occur. Patients who survive a severe exposure to nickel carbonyl may develop weakness and neurasthenic syndrome.

Data from occupationally exposed groups indicate that inhalation of nickel compounds may produce rhinitis, sinusitis and anosmia, and after a prolonged exposure, perforation of the nasal septum.

Acute ingestion of nickel compounds may cause nausea, vomiting, diarrhoea, headache, cough and shortness of breath. In severe cases, ingestion of large amounts of a nickel compound may cause death. Chronic oral exposure to nickel or nickel compounds has not been characterised in humans.

Dermal exposure to nickel salts can cause skin irritation. Nickel and its water soluble salts are potent skin sensitisers. Once sensitisation has occurred dermal exposure to even small amounts of nickel or nickel compounds can lead to an outbreak of contact dermatitis. In the past, prolonged dermal exposure of the public to nickel occurred widely from certain jewellery, earrings, watch straps etc. This was a major cause of skin sensitisation. Widespread exposure has now been largely eliminated by the Nickel Directive which places an upper limit (0.5 µg cm^{-2} $week^{-1}$) on the amount of nickel released from such products. This was implemented in the UK in 2000.

The International Agency for the Research on Cancer (IARC) concluded that nickel compounds are human carcinogens (Group 1). IARC also concluded that elemental nickel is a possible human carcinogen (Group 2B). No classification was given for nickel alloys.

Nickel carbonyl is classified in the EU as a reproductive toxicant (Risk Phrase R61 – 'may cause harm to the unborn child'). Several animal studies have reported malformations in the offspring of animals exposed to nickel carbonyl. Soluble salts such as the sulphate and the chloride are also similarly classified.

Kinetics and Metabolism

Nickel can be absorbed via inhalation, ingestion and to a very limited extent following dermal exposure. The absorption of nickel is related to the solubility of the compound, the general order of absorption being: nickel carbonyl > soluble nickel compounds > insoluble nickel compounds.

The extent of absorption following inhalation depends on particle size and the solubility of the compound. Smaller particles penetrate deeper into the respiratory tract than larger ones and therefore the relative absorption is greater. Smaller compounds are absorbed more quickly making them less available for mucociliary clearance and then being swallowed [1].

The presence of food in the stomach reduces the bioavailability of nickel. In an absorption study 27% of nickel sulphate given to humans in drinking water was systemically absorbed compared with 1% when it was given in food [1, 2].

Percutaneous absorption is minimal, but is clinically important in the development of contact dermatitis [1].

Human autopsy studies on individuals non-occupationally exposed to nickel found the highest concentrations of nickel in the lungs, thyroid and adrenal glands. Lower levels of nickel have been detected in the kidneys, heart, liver, brain, spleen and pancreas [2, 3]. Nickel crosses the placenta and has been found in breast milk [1].

The elimination of absorbed nickel predominately occurs via the urine, although some may be excreted in saliva, sweat, milk and tears. Nickel that is not absorbed from the gastrointestinal tract is excreted in the faeces [1]. A urinary elimination half life (for absorbed nickel) of 17 – 48 hours has been reported in a human oral exposure study [3].

Sources and Route of Human Exposure

Nickel occurs naturally in the earths crust and is ubiquitous in air, water, soil and the biosphere. The average concentration of nickel in the earth's crust is 0.008% [1, 2].

Nickel also exists as a number of compounds. Nickel compounds that are soluble in water include nickel chloride and nickel sulphate; insoluble nickel compounds include nickel oxide, nickel sulphide and nickel subsulphide [1]. Nickel carbonyl is a highly toxic, volatile liquid that has specialised industrial uses [4].

Nickel is emitted to the atmosphere from natural sources including windblown dust, volcanoes, vegetation, forest fires and meteoric dust [2]. The principle anthropogenic sources of nickel emissions to the atmosphere include the combustion of coal and oil, municipal incineration, steel and other nickel alloy production and electroplating [1, 2]. In urban areas, nickel levels in the ambient air range from 1-10 ng m^{-3}. In industrialised areas and large cities levels in the range of 110-180 ng m^{-3} have been recorded [3]. In polluted air, the main nickel compounds appear to be nickel sulphate, nickel oxides, nickel sulphides, and to a smaller extent, elemental nickel [1].

Food and cigarette smoke are the main sources of nickel exposure in the general public [3, 5]. Approximately 0.04-0.58 µg nickel is released with the mainstream smoke of one cigarette. Smoking 40 cigarettes a day may therefore lead to inhalation of 2-23 µg nickel [1]. The average daily intake of nickel from foodstuffs for an adult has been estimated to be

approximately 152 µg [5]. The general public may also be exposed to low levels of nickel by inhaling ambient air or by drinking water contaminated with nickel [2].
In the past, items such as less expensive jewellery, earrings, wristwatches etc were a common source of exposure to nickel (resulting in a high incidence of nickel sensitization). This exposure route has largely been eliminated by the 'Nickel Directive' which was implemented in the UK in 2000. A requirement of the Directive was that the upper limit for nickel release in articles which have direct or prolonged contact with the skin was 0.5 µg cm^{-2} $week^{-1}$ [6].

Exposure to nickel in the workplace may occur by dermal contact, by inhalation of aerosols, fumes, dusts or mists containing nickel or by inhalation of gaseous nickel carbonyl [7]. Occupational exposure to nickel is considered to be highest for individuals involved in production, processing and use of nickel [2]. Workplace exposure limits (WELs) for nickel and its inorganic compounds have been derived in the UK. The long-term exposure limit (LTEL) for water soluble nickel compounds is 0.1 mg m^{-3} (8 hour time weighted exposure (TWA) reference period) and the LTEL for nickel and water-insoluble nickel compounds is 0.5 mg m^{-3}. The short-term exposure limit (STEL) for nickel carbonyl is 0.24 mg m^{-3} (15 minute reference period) [8].

Health Effects of Acute / Single Exposure

Human Data

Inhalation

Nickel carbonyl is the most toxic nickel compound following acute exposure. The symptoms of acute exposure to nickel carbonyl occur in two stages, immediate and delayed [1, 9]. The immediate toxic effects of nickel carbonyl exposure are respiratory tract irritation and neurological symptoms. Initial symptoms include dizziness, frontal headache, nausea, vomiting, irritability and upper airway irritation [4, 5]. Following the immediate symptoms there is an asymptomatic period before the onset of the delayed pulmonary symptoms; similar to those of a viral pneumonia [1, 9]. Symptoms include chest pain, cough, dyspnoea, tachycardia, weakness and fever with leukocytosis. Pulmonary haemorrhage, cerebral oedema, toxic myocarditis, pulmonary oedema and pneumonitis may occur in severe cases [1, 4]. Neurasthenic syndrome and weakness may develop following a severe exposure to nickel carbonyl, and may persist for up 6 months [4].

There are limited data available on the acute effects of elemental nickel inhalation in humans. Death due to adult respiratory distress syndrome was reported in an individual who was exposed to elemental nickel at a concentration of 382 mg m^{-3} for 90 minutes [2, 5].

Ingestion

Only limited data are available on the effects of acute ingestion of nickel salts in humans.

Thirty-two industrial workers accidently ingested water contaminated with nickel sulphate and nickel chloride (1.63 g L^{-1}). Twenty of the workers rapidly developed symptoms including nausea, vomiting, diarrhoea, headache, cough and shortness of breath; all were asymptomatic within three days. Temporary elevated levels of blood reticulocytes (7 workers), urine-albumin (3 workers) and serum bilirubin (2 workers) were also reported. The nickel doses in the symptomatic workers were estimated to range from 7-36 mg kg^{-1} bw [1, 2].

A 2 year old child ingested approximately 570 mg kg^{-1} bw of nickel sulphate crystals and on admission to hospital she was somnolent with wide and unresponsive pupils, high pulse rate and pulmonary rhonchi. Cardiac arrest occurred 4 hours after ingestion. Gastrointestinal distress and muscular pain were also reported [1, 5].

Ingestion of a single dose of nickel salts has been reported to exacerbate vesicular hand eczema in nickel-allergic patients [1, 2, 5].

Dermal/ocular exposure

Primary skin irritation was observed when human skin was patch tested with a solution of nickel salts. A 10% aqueous solution of nickel chloride caused irritation when applied to the back. Irritation was observed when a 20% aqueous solution of nickel sulphate was applied to human forearm skin once a day for 3 days [1].

Animal and In-Vitro Data

General toxicity

The main target organ for nickel induced toxicity in animals is the respiratory tract [3]. The toxicity of nickel compounds appears to be related to the solubility of the compound. Several acute animal studies have reported soluble nickel sulphate as being the most toxic and insoluble nickel oxide the least toxic [2].

Inhalation

The 30 minute LC_{50} values for nickel carbonyl inhalation in cats, rats and mice were 0.19 mg L^{-1}, 0.24 mg L^{-1} and 0.067 mg L^{-1}, respectively. The pulmonary effects and lesions are comparable to those seen in cases of human nickel carbonyl poisoning. Pulmonary effects include oedema, intra-alveolar haemorrhage and fibrosis. Other lesions including congestion, oedema, focal haemorrhage, vacuolisation and mild inflammation in the brain, liver, kidney, adrenals, spleen and pancreas have been reported in laboratory animals acutely exposed to nickel carbonyl [1].

In an acute inhalation study, chronic inflammation was observed in rats exposed to nickel sulphate (0.7 mg m^{-3}) or nickel subsulphide (0.44 mg m^{-3}) 6 hours day^{-1} for 12 days in a 16 day period. Alveolitis was reported in rats exposed to 0.22 mg m^{-3} as nickel subsulphide 6 hours day^{-1} for 7 days. Acute lung inflammation was observed in rats exposed to nickel oxide at 7.9 mg m^{-3}. In mice, the lowest observed adverse effect levels (LOAELs) of 0.7, 1.83 and > 23.6 mg m^{-3} as nickel sulphate, nickel subsulphide and nickel oxide, respectively, were identified for chronic inflammation. These results suggest that mice are less sensitive than rats to the acute toxicity of nickel [2].

Atrophy of the nasal olfactory epithelium has been reported in rats exposed to nickel sulphate or nickel subsulphide [2]. Inhalation exposure to nickel chloride has resulted in immunological effects including alveolar macrophage alterations in laboratory animals [2].

Ingestion

Acute oral lethality studies suggest that soluble nickel compounds are more toxic than the less-soluble nickel compounds. Acute oral LD_{50} values of 46 and 39 mg kg^{-1} nickel sulphate were reported in male and female rats, respectively. In rats the oral LD_{50} values for the less soluble nickel compounds nickel oxide and subsulphide were >3,930 and >3,665 mg kg^{-1}, respectively [2].

The oral LD_{50} for nickel acetate has been reported as 350 mg kg^{-1}in the rat and 410 mg kg^{-1} in the mouse. Diarrhoea, distress and lethargy were noted 2 – 3 hours after exposure in animals which died [1].

Health Effects of Chronic / Repeated Exposure

Human Data

Inhalation

The respiratory tract is the primary site of toxicity following inhalation of nickel and its compounds. Rhinitis, sinusitis, asthma, chronic bronchitis, emphysema and nasal septal perforations have frequently been reported in individuals occupationally exposed to nickel or nickel compounds. Hyposmia or anosmia was also noted in many of the workers with sinusitis. Pulmonary changes with fibrosis were also observed in workers exposed to nickel dust or fumes[1, 5].

Ingestion

There are currently no data available on the effects of chronic ingestion to nickel or nickel compounds in humans.

Dermal / ocular exposure

Nickel and water soluble nickel salts are powerful skin sensitisers. Following sensitisation, dermal exposure to even small amounts of nickel or water soluble nickel salts can cause outbreaks of dermatitis [1].

Contact dermatitis is the predominant effect of nickel in the general population due, in the past, to prolonged dermal exposure from products such as jewellery, buttons and zips. The incidence of nickel sensitivity is higher in females (around 10% of the population) compared with males and has been associated particularly with ear piercing. Occupational induced contact dermatitis occurs in individuals who work in refining and electroplating industries [10].

Genotoxicity

Cytogenetic studies in workers involved in nickel crushing/roasting/smelting processes (mainly exposed to nickel oxide and nickel sulphide) and electrolysis (mainly exposed to nickel chloride and nickel sulphate) reported elevated levels of chromosome aberrations, mainly gaps). Chromosome aberrations, including elevated levels of gaps and breaks, were also seen in retired nickel workers from the same plant who had plasma nickel levels of 2 μg L^{-1} [1, 7].

Elevated levels of sister chromatid exchange and chromosome aberrations were reported in electroplating workers. No exposure or chemical data were provided [1]. In contrast, no effects were observed in workers in a nickel carbonyl production plant, although authors proposed this was due to low exposure due to adequate protective measures [1, 7].

Carcinogenicity

Increased risks of cancer of the lungs and nasal passages have been reported in workers exposed to nickel compounds (oxidic and sulphidic nickel) during roasting, sintering and calcining processes, in refineries in the UK, Norway and Canada. These processes have involved substantial exposure to insoluble nickel compounds such as nickel subsulphide and oxide, and also possibly to soluble salts such as nickel sulphate.

Soluble nickel is also associated with an increased risk of cancer to the lungs and nasal passages in, for example, electrolysis workers with estimated exposures of 1-2 mg m^{-3}. There is evidence to suggest it may enhance risks associated with exposure to less soluble nickel compounds [1, 7, 11].

The IARC concluded that there is sufficient evidence in humans for the carcinogenicity of nickel sulphate, and of the combinations of nickel sulphides and oxides encountered in the nickel refining industry. Nickel compounds are classified as carcinogenic to humans (Group 1) [7].

No significant increase in respiratory tract cancer was reported in users of elemental nickel powder or in workers involved in high-nickel alloy manufacture. IARC concluded that there is inadequate evidence in humans for the carcinogenicity of elemental nickel and nickel alloys. There was sufficient evidence in experimental animals for the carcinogenicity of elemental nickel hence it was classified as possibly carcinogenic to humans (Group 2B). There was limited evidence for carcinogenicity of nickel alloys in animals. IARC did not give any overall classification for nickel alloys [7].

Reproductive and developmental toxicity

There are no data available on the reproductive and developmental effects of nickel and its compounds in humans.

Animal and In-Vitro Data

Inhalation

A number of studies have investigated the effect of chronic inhalation exposure to nickel or nickel compounds in laboratory animals. The target organ for nickel toxicity is the lung.

Lung irritation was observed in rats, mice and guinea pigs exposed to elemental nickel dust at 15 mg m^{-3}. In rats, nasal sinus inflammation and ulcers were also observed [1].

Rats and mice exposed to 0.06 mg m^{-3} nickel sulphate, 0.11 mg m^{-3} nickel subsulphide and 0.4 mg m^{-3} nickel oxide six hours per day, five days per week for 16 or 90 days resulted in inflammation of the lungs, fibrosis and increased lung weight. Toxicity was directly related to the compounds solubility [5].

The toxicity of nickel oxide, nickel sulphate and nickel subsulphide was also investigated in a two year inhalation study in rats and mice. Chronic lung inflammation was observed in rats and mice at the lowest administered dose of nickel oxide (0.5 mg m^{-3}) and nickel subsulphide (0.11 mg m^{-3}) in both species. Nickel sulphate produced lung effects in both species at doses of 0.06 mg m^{-3} and above [5].

Inflammatory changes in the nasal mucous membranes and the trachea, bronchitis and slight fibrosis in the lung were reported in rabbits exposed to 100 mg m^{-3} nickel graphite dust for 3 hours a day, 5 days a week for 6 months [1].

Rats exposed to nickel oxide (53 mg m^{-3} nickel oxide) for the life span developed emphysema and pneumoconiosis. Abscesses and metaplastic changes were observed in rats exposed to nickel subsulphide for 78 weeks [1].

Other effects reported in laboratory animals exposed to nickel or its compounds include atrophy of the nasal epithelium, reduction in body weight gain and immunological and lymphoreticular effects [1, 2].

Ingestion

The main toxic effects observed following long-term oral exposure of laboratory animals to nickel compounds are on the lungs and kidneys.

Mice exposed to ≥108 mg kg^{-1} bw day^{-1} as nickel sulphate in drinking water for 180 days developed minor renal tubular damage. Significant decreases in urinary glucose and urine volume were reported in rats exposed to 14.4 or 28.8 mg bw kg^{-1} day as nickel sulphate in drinking water for 13 days. Increases in blood urea were also observed in rats exposed to 28.8 mg bw kg^{-1} [2].

In a two year dietary study rats were given 0, 5, 50 or 125 mg kg^{-1} bw day^{-1} nickel sulphate. A significant reduction in body weight gain was noted in rats given ≥ 50 mg kg^{-1} day^{-1}. No histopathological changes were observed; therefore the NOAEL was taken to be 5 mg kg^{-1} bw day^{-1} [5].

In a 90 day gavage study in rats administered nickel chloride the NOAEL was found to be 5 mg kg^{-1} bw day^{-1}. Effects on body weight, on organ weight and on the nervous system were reported at doses of 35 and 100 mg kg^{1} bw day^{-1} [5].

Pneumonitis was reported in rats administered 8.6 mg kg^{-1} bw day^{-1} as nickel chloride by gavage for 91 days. Significant increases in absolute and relative lung weights were reported in rats exposed to 28.8 mg kg^{-1} bw day^{-1} in drinking water for 13 weeks. Dogs exposed to 22.5 mg kg^{-1} bw day^{-1} as nickel sulphate in the diet for 2 years developed emphysema, bronchiolectasis and cholesterol granulomas [2].

A reduction in body weight gain has also been reported in laboratory animals following chronic oral exposure to nickel compounds [2].

Dermal/ocular exposure

Skin atrophy, acanthosis, and hyperkeratinisation were observed in rats following repeated skin application of 40 – 100 mg kg^{-1} as nickel sulphate [1].

Experimental sensitisation has been observed in laboratory animals, but only under special conditions rather than using more routine methods [1].

Genotoxicity

Chromosomal aberrations were induced in the bone marrow cells of Chinese hamsters and Swiss mice administered nickel chloride by intraperitoneal injection [1, 7].

Nickel compounds produced negative results in the majority of bacterial mutation assays reported [1, 7].

In one study, elemental nickel did not induce chromosomal aberrations in cultured human peripheral lymphocytes [7].

Nickel subsulphide induced cell transformation and increased the frequency of sister chromatid exchange but did not cause gene mutation in cultured human cells. In animal cells *in-vitro* nickel subsulphide and nickel sulphide induced cell transformation, gene mutation

and DNA damage; nickel sulphide also induced sister chromatid exchange and chromosome aberrations [7].

Soluble nickel compounds have generally given positive results in human and animal *in-vitro* assays. In human cells *in-vitro,* nickel sulphate induced cell transformation and chromosome aberrations, nickel sulphate and nickel chloride increased the frequency of sister chromatid exchange Nickel sulphate and nickel chloride have been reported to cause cell transformation, chromosome aberrations, sister chromatid exchange and gene mutations in cultured animal cells. Nickel chloride has also been reported to induce DNA damage in animal cells [1, 7].

In summary nickel compounds are generally inactive in bacterial assays but positive results were obtained in mammalian cell assays. There is a lack of information from published *in-vivo* assays.

Carcinogenicity

A number of studies have investigated the carcinogenicity of nickel and its compounds in experimental animals [7].

Several studies reported that tumours were induced at the site of injection or implantation of a nickel compound. In these studies nickel subsulphide was the most potent carcinogen [7].

An inhalation study reported an increased incidence of lung tumours (adenomas, adenocarcinomas, squamous cell carcinomas and fibrosarcoma) in rats exposed to nickel subsulphide [2, 7]. In a 2 year NTP inhalation bioassay in mice and rats exposed to nickel subsulphide, nickel oxide or nickel sulphate no increased incidences of tumours were reported in mice exposed to any of the compounds or rats exposed to nickel sulphate. However, significant increases in lung adenomas and carcinomas were observed in rats exposed to the less-soluble nickel compounds (nickel oxide and nickel subsulphide). Exposure to nickel oxide or subsulphide also caused significant increases in the incidence of adrenal pheochromocytomas in rats [2, 3].

In 1990, IARC concluded that there is sufficient evidence in experimental animals for the carcinogenicity of elemental nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulphides. IARC also concluded that there is insufficient evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides and nickel telluride. The evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulphide and nickel titanate was considered to be inadequate [7].

Reproductive and developmental toxicity

A number of multigeneration reproductive studies have been carried out in rats, administered soluble nickel salts in water. The main effect noted was increased neonatal mortality at exposures producing maternal toxicity. In some studies there was also some evidence for effects at levels that did not produce maternal toxicity [5].

In a two generation study using nickel chloride in drinking water, reduced numbers of viable pups and increased mortality was seen at doses above 30.8 mg kg^{-1} bw day^{-1}, which were also associated with maternal toxicity. There was equivocal evidence at lower dose levels. In another two generation study in rats, however, increased perinatal mortality was seen in the second litter at the lowest dose investigated (1.3 mg kg^{-1} bw day^{-1}), but there was no clear dose response in this study. In a three generation study in rats, increased neonatal mortality was seen at doses estimated to be approximately 0.5 mg kg^{-1} bw day^{-1} [5].

In a more recent gavage study, an increased post-implantation loss and pup mortality were seen in rats given nickel sulphate at 2.2 mg kg^{-1} bw day^{-1} [5].

A series of studies reported malformations in the offspring of rats and hamsters exposed to nickel carbonyl by inhalation or injection before, or a few days after implantation. Malformations reported include anophthalmia, microphthalmia, cystic lungs, fused rib, cleft palate, exencephaly and hydronephrosis [1, 7].

Both nickel carbonyl and a number of soluble nickel salts (sulphate, chloride, nitrate and carbonate) are classified in the EU as toxic to reproduction Category 2 on the basis of their developmental toxicity (and are required to be labelled 'may cause harm to the unborn child') [12].

There are limited data from investigating nickel compounds specifically in developmental toxicant studies.

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Metal-ion tolerance in *Escherichia coli*: analysis of transcriptional profiles by gene-array technology

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***Escherichia coli* was adapted to grow in medium containing substantially elevated concentrations of either Zn(II), Cd(II), Co(II) or Ni(II). Whole-genome transcriptional profiles were generated from adapted strains and analysed for significant alteration in transcript abundance with reference to a wild-type strain. Similar alterations in specific message levels were observed for strains adapted to the four metal ions. One unexpected trend was the increase in transcript level of genes involved in transposition of IS elements, particularly *insA*. Subsequent expression of *insA-7* from a heterologous promoter in *E. coli* conferred tolerance to Zn(II).**

Keywords: *Escherichia coli*, metal ion, adaptation, gene array, *insA*

INTRODUCTION

Many mechanisms solely responsible for specific metal-ion resistance in prokaryotes have been described at the molecular level (recently reviewed by Nies, 1999; Xu *et al.*, 1998). The effect of metal-ion stress on microbial cells/communities has been investigated and suggests that individual strains adapt to elevated metal-ion concentrations; however, no analysis of the resultant strains was initiated at the molecular level (reviewed by Giller *et al.*, 1998; Kelly *et al.*, 1999). Limited genetic analysis has been performed for a Cd(II)-tolerant strain of *Synechococcus* PCC 7942 that demonstrated an increase in the chromosomal gene copy number of *smtA*, which encodes a prokaryotic metallothionein capable of binding Cd(II) and other divalent metal ions (Gupta *et al.*, 1992). In addition, the regulatory gene *smtB* was shown to carry a deletion rendering the *smt* repressor protein non-functional (Gupta *et al.*, 1993). The molecular bases for the increase in Cd(II) tolerance in *Synechococcus* are almost certainly more extensive than the amplification/deletion within the *smt* locus, although at the time of the original experiments further characterization of other chromosomal alterations was not possible. As far as we are aware, genomic analysis of metal tolerance in bacterial cells has not been undertaken.

The recent advent of array technology has allowed the global study of all 4255 genes in *Escherichia coli*. This work details the investigation of metal-ion tolerance in *E. coli* adapted to Zn(II), Cd(II), Co(II) and Ni(II), and the results demonstrate that the transcript abundance of a range of genes was altered in tolerant strains. In particular, the transcript level of *insA* was increased in Zn(II)-, Cd(II)- and Co(II)-adapted strains. The subsequent expression of *insA*-7 from a heterologous promoter conferred Zn(II) tolerance on *E. coli*.

METHODS

Bacterial strains and plasmids. All experiments were carried out using as host *Escherichia coli* TG1 (described in Sambrook *et al.*, 1989) [K12, *lac–pro supE thi hsdD5* (F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} *lacZ* M15)] or *E. coli* TG2 (described in Sambrook *et al.*, 1989) (which is *E. coli* TG1 carrying the *recA*::Tn*10* mutation). pBAD24 (Guzman *et al.*, 1995) was used for *insA*-7 expression. All strains were cultured in Luria–Bertani medium and antibiotics were added to the medium at standard concentrations unless otherwise specified (Sambrook *et al.*, 1989).

Adaptation of *E. coli* TG1 to metal-ion tolerance. A 10 ml volume of LB medium was inoculated with 150 µl of an overnight culture of *E. coli* TG1 and grown with shaking for 1·5 h, before 200 µl aliquots were transferred into a 96-well plate containing an increasing range of metal-ion concentrations of Zn(II) ($ZnSO_4$), Cd(II) ($CdSO_4$), Co(II) ($CoSO_4$) and Ni(II) ($NiSO_4$). The plates were incubated at 37 °C for 24–48 h with shaking (Camlab Microtherm; 500 r.p.m.) and the OD_{600} of cell cultures was measured (Molecular Devices Thermo max microplate reader). A 150 µl volume was taken from the well containing the highest concentration of each individual metal ion where the OD_{600} was $\geqslant$0·100, and this was used to inoculate 10 ml LB medium for further adaptive incubation. This stepwise adaptation generated strains with

increased tolerance to Zn(II) (strain *E*Zn), Cd(II) (strain *E*Cd), Co(II) (strain *E*Co) and Ni(II) (strain *E*Ni). Adaptation was concluded when cultures reached a stable tolerance maximum over two serial culture steps. The resultant cultures were either diluted 50% (v/v) with glycerol for storage or used for the isolation of RNA.

Measurement of metal-ion tolerance (MICs). A 30 ml volume of LB medium was inoculated with the individual strains of *E. coli* TG1, *E*Zn, *E*Cd, *E*Co and *E*Ni, and grown with shaking for 1 h at 37 °C. A 200 µl sample of each culture was transferred into separate wells of a microtitre plate which contained increasing ranges of meral-ion concentrations, and incubated at 37 °C for 24 h with shaking (Camlab Microtherm; 500 r.p.m.). The OD_{600} of cell cultures was then measured (Molecular Devices Thermo max microplate reader). MICs for each strain were measured for the four metal ions Zn(II), Cd(II), Co(II) and Ni(II). In some cases it was impossible to accurately determine an MIC as the strains grew in medium in which the given metal ion was at the limit of solubility.

Whole-genome transcriptional analysis. *E. coli* Panorama arrays were used throughout this work (Sigma-Genosys). Metal-ion-tolerant *E. coli* strains were grown ro stationary phase in LB containing the maximum permissive concentration of the appropriate metal salt. RNA was extracted using a Qiagen RNeasy kir according to the manufacturer's instructions. cDNA probes were generared as described by Sigma-Genosys, using primers and reverse transcriptase provided. Hybridized probes were visualized by autoradiography using a phosphorimager (Bio-Rad, Personal FX). Hybridization intensity was quanrified and compared using Quantity One (Bio-Rad) and Excel (Microsoft). Transcriptional profiles for each strain of cells were compared to that of the wild-rype strain grown to stationary phase in the absence of meral ions. LB medium was used to allow future array experiments involving alternative challenges to be comparable with this data set.

Amplification of the *insA-7* coding region and plasmid construction. All DNA manipulations were carried out according to Sambrook *et al.* (1989). The *insA-7* coding region was amplified from rhe Co(II)-tolerant strain *E*Co using primers A7(V)N-term (5′-CGGAATTCTGCGTGGCTTCC-ATTTCCATCAGATGTCC-3′) and A7C-term (5′-GCTC-TAGACGCTGACGTGATTTAGCACCGACG-3′) (synthesized by Gibco-BRL). The nucleotide sequence of rhe amplified *insA* was determined and was idenrical to that reported, except for one base change (+150 from start codon: C to A). This change does not affect the primary amino acid sequence. The derivative of pBAD24 (Guzman *et al.*, 1995) carrying the *insA*-7 coding region was created by digesting the PCR product with *Eco*RI and *Xba*I and ligating together with identically cut pBAD24 to generate pBAD*insA*-7.

***insA-7* expression.** Overnight culrures of TG2(pBAD24) or TG2(pBAD*insA*-7) were used to inoculate 20 ml fresh LB medium containing arabinose at a final concentration of 0·5% (w/v). A 200 µl sample of each culrure was transferred into separate wells of a microtitre plate containing increasing concentration ranges of Zn(II), Cd(II), Co(II) or Ni(II). The cultures were incubated at 37 °C with shaking, and the OD_{600} was measured (Molecular Devices Thermo max microplate reader) every 1 or 2 h over a 30 h period. Carbenicillin was added to medium to allow selection of pBAD24 and pBAD*insA*-7 which carry the *bla* marker gene.

RESULTS AND DISCUSSION

Adaptation of *E. coli* to metal tolerance

E. coli TG1 was grown in LB medium supplemented with a range of either Zn(II), Cd(II), Co(II) or Ni(II) and the MICs were determined as 2·2, 1·2, 1·7 and 4·0 mM, respectively (Fig. 1). The MIC values for the four metal ions were also determined for derivative strains individually adapted for growth in elevated concentrations of Zn(II) (strain *E*Zn), Cd(II) (strain *E*Cd), Co(II) (strain *E*Co) and Ni(II) (strain *E*Ni). Despite the diminished solubility of metal ions in a rich medium, a clear increase in tolerance was observed for all the adapted strains. *E*Zn was tolerant to Zn(II) at 9·0 mM (Fig. 1) and also exhibited slight cross-tolerance to Co(II) and Ni(II) but sensitivity to Cd(II) (data not shown). *E*Cd exhibited an MIC to Cd(II) of 4·0 mM (Fig. 1) and this strain showed increased tolerance to Co(II) and Ni(II) (data not shown). *E*Co is dramatically increased in tolerance, exhibiting growth at over 23·0 mM supplementary Co(II) (Fig. 1) and showing an increase in tolerance to Ni(II) (data not shown). The MIC of *E*Ni was 19·0 mM (Fig. 1) and significant cross-tolerance to Co(II) was observed with an increase in sensitivity to Cd(II) (data not shown). That metal-ion cross-tolerance/dependence was observed is unsurprising given the chemical similarities that exist between these divalent cations.

Transcriptional analysis of adapted strains

Transcriptional profiles were generated using macroarrays (Sigma-Genosys) representing all 4255 ORFs present in the *E. coli* genome (Blattner *et al.*, 1997). The genes of interest which were significantly modulated (more than two standard deviations from the mean) are detailed (Tables 1 and 2). For brevity, not all significant results are shown here (complete listings can be accessed via URL http://www.cf.ac.uk/biosi/staff/people/morby.html).

Analysis of genes for which transcriptional levels are decreased (Table 1) reveals a significant commonality. Of those with a known function, *yfiA* (translational stimulator; Bylund *et al.*, 1997), *tufA*/*tufB* (EF-Tu; reviewed by Weijland *et al.*, 1992) and *yjbC* (putative pseudouridylate synthase, shows similarity to *rsuA*; Wrzesinski *et al.*, 1995) encode proteins involved in translation; this adaptation may comprise a general response to stress or may merely be a function of lower growth rates in the presence of a toxic agent. In addition, *tnaA* (tryptophanase; Deeley & Yanofsky, 1981) and *aspA* (aspartase; Guest *et al.*, 1984) are reduced, suggesting the cell is less catabolic in nature.

Consistent with tolerance to cations, *ompC* and *ompA*, which encode outer-membrane porins (Ried *et al.*, 1990), are reduced in most of the adapted strains. b0795, which shows similarity to CzcB (a component of a cation efflux pump; Nies *et al.*, 1989), is reduced in all four strains. Analysis of the b0795-encoded sequence shows the presence of a signal peptide, suggesting it is periplasmic in location. In addition, the operon which contains

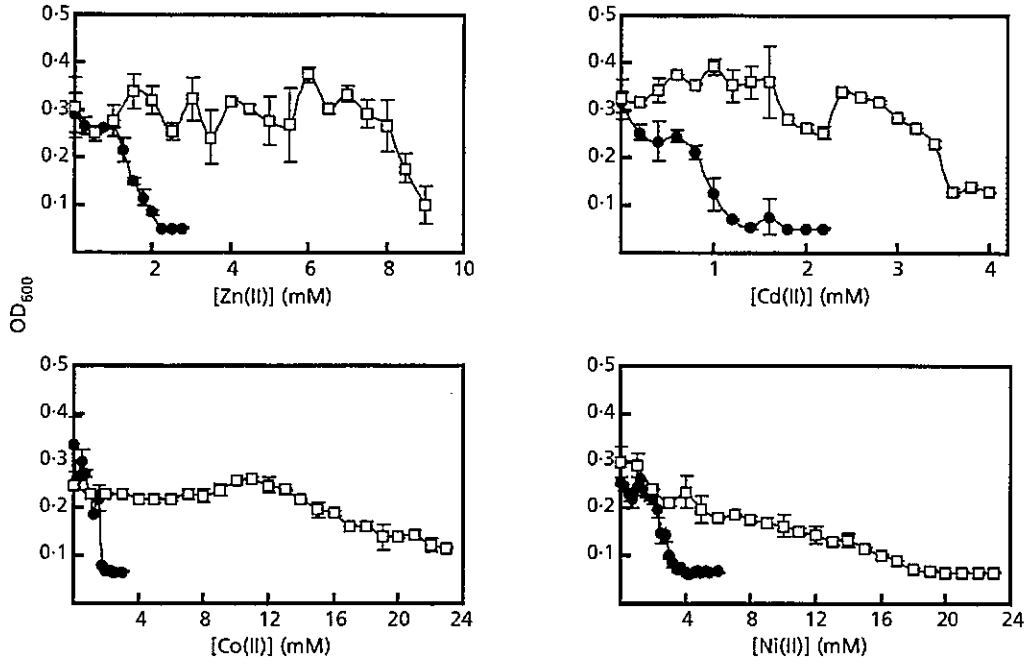

Fig. 1. Liquid MICs for Zn(II), Cd(II), Co(II) and Ni(II), shown for *E. coli* TG1 adapted for tolerance to Zn(II) (strain *E*Zn), Cd(II) (strain *E*Cd), Co(II) (strain *E*Co) and Ni(II) (strain *E*Ni), respectively. The OD_{600} was determined for each strain (□); wild-type *E. coli* TG1 is shown on each graph as a comparison (●). The graphs show OD_{600} against metal-ion concentration. Assays were performed in triplicate and the SDs are shown.

Table 1. Transcriptional profiles of metal-ion-tolerant strains: genes of interest for which transcriptional levels were decreased are listed

The fold decrease in transcript abundance is shown for each gene in each strain where appropriate.

Gene	Gene product description	Fold decrease in strain:			
		*E*Zn	*E*Cd	*E*Co	*E*Ni
yfiA	Putative translational stimularor	9·7	16·2	11·4	15·0
tnaA	Tryptophanase	9·2	15·4	9·8	15·3
tufB	Elongation factor EF-Tu (duplicate gene)	7·3	11·4	12·0	11·5
yjbC	Putative pseudouridylate synthase	5·6	5·0	10·2	10·4
aspA	Aspartase	4·0	8·8	7·9	7·7
tufA	Protein chain elongation factor EF-Tu (duplicate of *tufB*)	3·4	5·5	8·9	12·1
b0795 (f332)	Putative periplasmic protein, sequence similarity to *czcB*	6·7	4·5	6·9	7·0
ompC	Outer-membrane protein C precursor	5·4	6·4	4·8	3·9
ompA	Outer-membrane protein A	5·0	3·5		5·8

b0795 also contains genes whose products are similar to ABC-transporter proteins. It is possible that this operon is involved in cation import and therefore it is consistent for one or more components to be diminished in metal-adapted cells.

Genes which show increased transcriptional levels (Table 2) also have some commonality between strains. Some induced genes have a functional association with metal cations, including *nikD* [Ni(II) export; Navarro *et al.*, 1993], *yfeC* [putative chelated-Fe(II) export; Bearden *et al.*, 1998], *yeaJ* (shows sequence similarity to *hmsT*, a putative regulator of haem storage; Jones *et al.*, 1999) and *bis*C (biotin sulfoxide reductase, binds molybdenum; Pierson & Campbell, 1990). These gene products could be involved in the chelation of excess cytosolic metal ions which may generate tolerance or perhaps represent compensatory alterations which preserve cation [e.g. Mn(II) or Fe(II)] metabolism in the presence of excess Zn(II), Cd(II), Co(II) or Ni(II).

The majority of the characterized genes identified in this

Table 2. Transcriptional profiles of metal-ion-tolerant strains: genes of interest for which transcriptional levels were increased are listed

The fold increase in transcript abundance is shown for each gene in each strain where appropriate.

Gene	Gene product description	Fold increase in strain:			
		*E*Zn	*E*Cd	*E*Co	*E*Ni
insA-7	Insertion element IS*1* protein InsA	3·5	3·8	2·2	
insA-6	Insertion element IS*1* protein InsA	3·5	3·4	3·8	
insA-1	Insertion element IS*1* protein InsA		3·3	3·6	
insA-2	Insertion element IS*1* protein InsA	3·3		3·0	
insA-5	Insertion element IS*1* protein InsA	4·0	6·6		
insB-1	Insertion element IS*1* protein InsB	2·6		4·2	
insB-6	Insertion element IS*1* protein InsB	2·8		3·1	
nikD	Nickel transport ATP-binding protein NikD	8·2	21·5		
yfeC	Putative chelated Fe(II)-export protein	8·0	20·1		
bisC	Biotin sulfoxide reductase			3·1	2·9
yeaJ	Putative haem storage regulator	3·1		4·3	

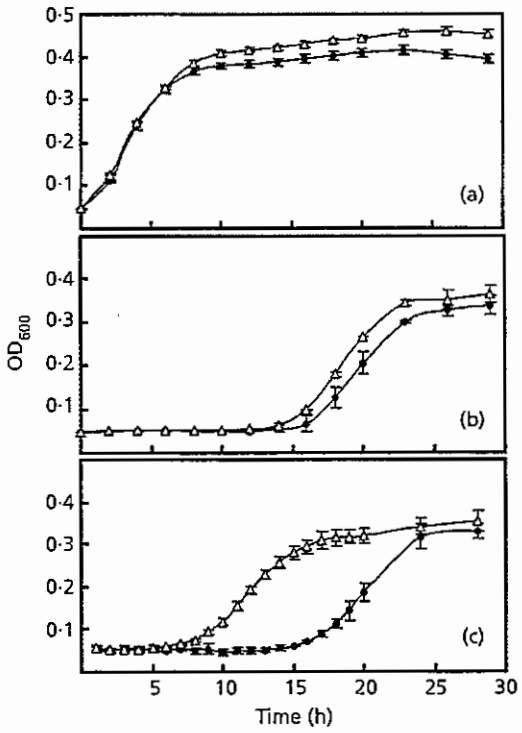

Fig. 2. Graphs showing growth curves for TG2(pBAD24) (●) and TG2(pBAD*insA*-7) (△). (a) Growth medium containing no additional metal ions. (b) Growth medium containing 0·6 mM Cd(II). (c) Growth medium containing 1·0 mM Zn(II). The graphs show OD_{600} against time. Assays were performed in triplicate and the SDs are shown.

experiment encode proteins involved in the transposition of IS*1*, *2*, *3*, *5* and *30* (see data at URL http://www.cf.ac.uk/biosi/staff/people/morby.html). It is well documented that IS genes may be induced by cellular stress and it is hypothesized that the movement of IS elements is capable of increasing genetic diversity (Naas *et al.*, 1994).

The most consistently induced genes are those for *insA* (IS*1* transposition), for which transcripts are elevated in three out of the four strains. It is impossible to determine from array analyses which of the *insA/B* sequences were induced, given the close sequence similarity within the gene families, which may have resulted in cross-hybridization during the experiment. The repeated increase in transcript abundance of *insA* genes led to the investigation of metal tolerance in a wild-type strain expressing *insA*-7 from a heterologous promoter.

Increase in metal tolerance by overexpression of *insA-7*

E. coli TG2 carrying pBAD*insA*-7 was generated in which gene expression was controlled by the level of arabinose in the medium. *insA*-7 was used since all *insA* sequences are almost identical and this gene has no apparent associated *insB*. When grown in medium containing arabinose, but no additional metal ions, TG2(pBAD*insA*-7) showed a slight growth advantage over TG2(pBAD24) (Fig. 2a). In contrast, when grown in the presence of arabinose plus 0·6 mM Cd(II) and 1·0 mM Zn(II), TG2(pBAD*insA*-7) shows a slight increase in tolerance to Cd(II) but a marked increase in tolerance to Zn(II) (Fig. 2b, c). No increase in tolerance to Co(II) or Ni(II) was observed (data not shown). The *insA*-dependent increase in tolerance to Zn(II) is consistent with the observation that *E*Zn showed increases in transcript abundance for genes encoded by IS*1*.

InsA alone binds to p*insL* to negatively auto-regulate and inhibit transposition of IS*1* (Zerbib *et al.*, 1987; Machida & Machida, 1989; Matsutani, 1997). The

heterologous expression of *insA*-7 should therefore reduce transposition of IS*1* in TG2(pBAD*insA*-7), which suggests that the metal-tolerance phenotype is directly conferred by an increase of InsA-7 within the cell. This result contradicts the dogma that IS elements cannot directly enhance the fitness of their host, but merely carry genes for transposition functions (Blot *et al.*, 1993). Analysis of the InsA-7 primary sequence shows the presence of two cysteine-X-X-cysteine motifs which are known to bind metal ions in both prokaryotic and eukaryotic proteins containing HMA motifs (GXXC-XXC) (Bull & Cox, 1994); such motifs also occur in group I, II and III metallothioneins (reviewed by Kille *et al.*, 1994). The CXXC motifs in InsA-7 may enable this protein to bind metal ions in the cell cytosol. Given the spacing between the two CXXC motifs it is possible that these proteins bind to DNA using a Zn(II)-finger motif rather than the helix–turn–helix motif previously identified by weak similarity (24% identity) to known helix–turn–helix motifs (Zerbib *et al.* 1987).

Concluding remarks

In response to toxic concentrations of Zn(II), Cd(II), Co(II) or Ni(II), *E. coli* exhibited varying degrees of tolerance (3–14-fold greater than wild-type) both to the adaptive metal and its congeners. All of the adapted strains showed similar patterns of diminished gene expression, with particular bias towards genes whose products are involved in translation. No single gene whose expression increased was common to the four adapted strains, but surprisingly *insA* was increased in the Zn(II)-, Cd(II)- and Co(II)-adapted strains. Subsequent expression of *insA*-7 in *E. coli* demonstrated that this gene can confer tolerance to Zn(II), suggesting that it may be capable of binding divalent metal ions by virtue of two paired cysteine motifs. This study shows the utility of an array-based approach to functional genomics in *E. coli* and the capacity for the generation of novel avenues of research using these techniques.

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Biochemistry

The *zntA* gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase

(zinc transport/zinc resistance/cadmium resistance)

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ABSTRACT **The first Zn(II)-translocating P-type ATPase has been identified as the product of *o732*, a potential gene identified in the sequencing of the *Escherichia coli* genome. This gene, termed *zntA*, was disrupted by insertion of a kanamycin gene through homologous recombination. The mutant strain exhibited hypersensitivity to zinc and cadmium salts but not salts of other metals, suggesting a role in zinc homeostasis in *E. coli*. Everted membrane vesicles from a wild-type strain accumulated ^{65}Zn(II) and ^{109}Cd(II) by using ATP as an energy source. Transport was sensitive to vanadate, an inhibitor of P-type ATPases. Membrane vesicles from the *zntA*::*kan* strain did not accumulate those metal ions. Both the sensitive phenotype and transport defect of the mutant were complemented by expression of *zntA* on a plasmid.**

All living organisms require zinc for continued growth and development. The numerous functions include both structural and catalytic roles in a number of enzymes (1–3). However, in excess zinc is toxic, so both eukaryotes and prokaryotes have developed mechanisms to prevent overaccumulation of zinc, for example induction of metallothioneins that sequester zinc (4, 5). Another mode of zinc regulation is likely to be at the level of transporters that facilitate zinc influx during deficiency and efflux during excess. Despite the obvious importance of zinc homeostasis, little is known about these transporters at the molecular level (6–10).

P-type ATPases form a large family of cation-transporting membrane proteins, with more than 50 members identified to date (11). A recently identified subfamily of putative soft metal P-type ATPases has been implicated in metal homeostasis (11–13). Representative members of this subfamily include bacterial enzymes such as CadA (14–16), CopA and CopB (17), CtaA (18), and eukaryotic Cu(I)-transporting ATPases such as the Menkes (19) and Wilson disease-associated proteins (20). Four ORFs potentially encoding soft metal-translocating ATPases were identified in the *Escherichia coli* chromosome: HRA1 (731 residues; accession no. U16658) (21), HRA2 (721 residues; accession no. U16659) (21), a probable copper-translocating ATPase (834 residues; accession no. U58330) (S. Das, E. Chuang, C. Vulpe, J. Goldman, and J. Gitschier, unpublished data), and ORF732 (732 residues; accession no. P37617) (22).

The most striking feature of soft metal P-type ATPases is the presence of 1–6 motifs, GXXCXXC or (M/H)XXMDH(S/G)XM, at the N terminus of the molecule that are putative metal-binding domains (12, 13). Differences in the number of times this metal-binding motif is repeated provide the basis for the enormous variation in mass of these proteins. In addition, members of this group have either CPC or CPH in a putative transmembrane helix that might be part of the cation channel. However, neither the cationic substrate nor the physiological function can he deduced from the primary structure. Only two members of this subgroup have been demonstrated to transport metals: CadA transports cadmium ions (15), and CopB transports copper and silver ions (23). Recently peptides derived from Menkes and Wilson proteins have been demonstrated to bind Cu(I) (24).

E. coli is intrinsically tolerant to high levels of zinc and cadmium. We predicted that ORF732, the closest homolog to CadA, would be responsible for resistance by catalyzing the active efflux of zinc or cadmium. In this report we disrupted the gene by insertion of a kanamycin gene. The resulting strain exhibited hypersensitivity to zinc and cadmium that could be complemented by *o732* (renamed *zntA*) on a plasmid. Everted membrane vesicles from the wild-type but not the disrupted strain exhibited ATP-dependent ^{65}Zn(II) and ^{109}Cd(II) accumulation by using ATP as an energy source. Because everted membrane vesicles have an orientation opposite to that of intact cells, accumulation into the vesicles is equivalent to extrusion from the cells. The Zn(II) transport defect of the mutant could similarly be restored by expression of *zntA* on a plasmid. Transport was inducible by zinc and inhibited by orthovanadate, an inhibitor of P-type ATPases. This is the first demonstration of Zn(II) transport by a P-type ATPase. Considering the importance of zinc homeostasis, we would predict the existence of a homolog of ZntA for zinc metabolism in humans, with inheritable metabolic diseases perhaps as severe as the Menkes and Wilson diseases.

MATERIALS AND METHODS

Growth of Cells. Cells of *E. coli* were grown in Luria–Bertani (LB) medium (25) at 37°C. Ampicillin (50 μg/ml), kanamycin (50 μg/ml), tetracycline (15 μg/ml), isopropyl β-D-thiogalactopyranoside (0.1 mM), and 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (80 μg/ml) were added as required. The basal salts medium used for the determination of metal sensitivity was described by Poole *et al.* (26), except that zinc was omitted. For membrane preparation cells were grown overnight at 37°C in 20 ml of LB and diluted 50-fold in prewarmed medium. At an optical density of 0.8 at 600 nm the cultures were induced with 1 mM $ZnSO_4$ for 1 h. Controls were not induced.

Strain Construction and Plasmids. Plasmid DNA propagation, restriction enzyme treatment, ligation, and transformation were performed by minor modifications of published procedures (25). Plasmid pCGR2 was constructed by PCR amplification of the *zntA* gene, starting 97 bp upstream of the start codon and ending 78 bp downstream. The oligonucleotide

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Abbreviation: LB, Luria–Bertani.

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primers used for PCR were 5′-ATCGTCCGCTCGCTGTA-TCTCT-3′ and 5′-CCGCCTTTTCCCCTCACCCTAACC-3′. The PCR product was cloned into plasmid pGEM-T (Promega). Plasmid pCGR2 was then digested with *Sna*BI, which cuts at a unique site within *zntA*. The kanamycin resistance gene from plasmid pUC4K (Pharmacia) was excised as a *Sal*I fragment, and the ends were made blunt by using the Klenow fragment of *E. coli* DNA polymerase I. This gene was ligated with *Sna*BI-digested pCGR2, generating plasmid pCGR9. To disrupt the chromosomal *zntA* gene, pCGR9 was linearized with *Nco*I and *Sst*I, and the linear DNA was transformed into the *recD* strain JCB499 (27). Homologous recombination of the kanamycin resistance gene into *zntA* was confirmed by PCR with the above primers. The *zntA*-disrupted gene was transferred to strain W3110 by generalized transduction with P1 bacteriophage, with selection for kanamycin resistance.

Determination of Metal Sensitivity. Inhibition by metal salts was tested both in liquid and solid media. The minimal inhibitory concentration (MIC) is the concentration at which no growth was detected. Cells were grown overnight and streaked to single colonies on LB plates containing varying concentrations of metal salts. Growth was monitored after 24 h at 37°C. In liquid culture, strains were grown overnight, diluted 1:100 in liquid medium containing the varying concentrations of metal salts, and incubated for 24 h at 37°C with shaking. Growth was monitored from the absorbance at 600 nm.

Transport Assays. Everted membrane vesicles were prepared essentially as described previously and stored at −70°C until use (28). Transport assays were performed at room temperature. Unless otherwise noted, the reaction mixture (1 ml) contained 20 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane, pH 6.0, 0.2 M KCl, 0.25 M sucrose, 1 mM 2-mercaptoethanol, 0.8–1 mg of membrane protein, either 5 mM Na_2ATP or ADP, and either 10 μM $^{65}ZnSO_4$ or ^{109}Cd acetate (1.25 μCi/ml). The reaction was initiated by addition of 5 mM $MgSO_4$. At intervals, 0.1-ml samples were withdrawn, filtered through nitrocellulose filters (0.22-μm pore size, Whatman), and washed with 5 ml of the same buffer containing 10 mM $MgSO_4$ and either 20 mM $ZnSO_4$ or cadmium acetate. The filters were dried, and the radioactivity was

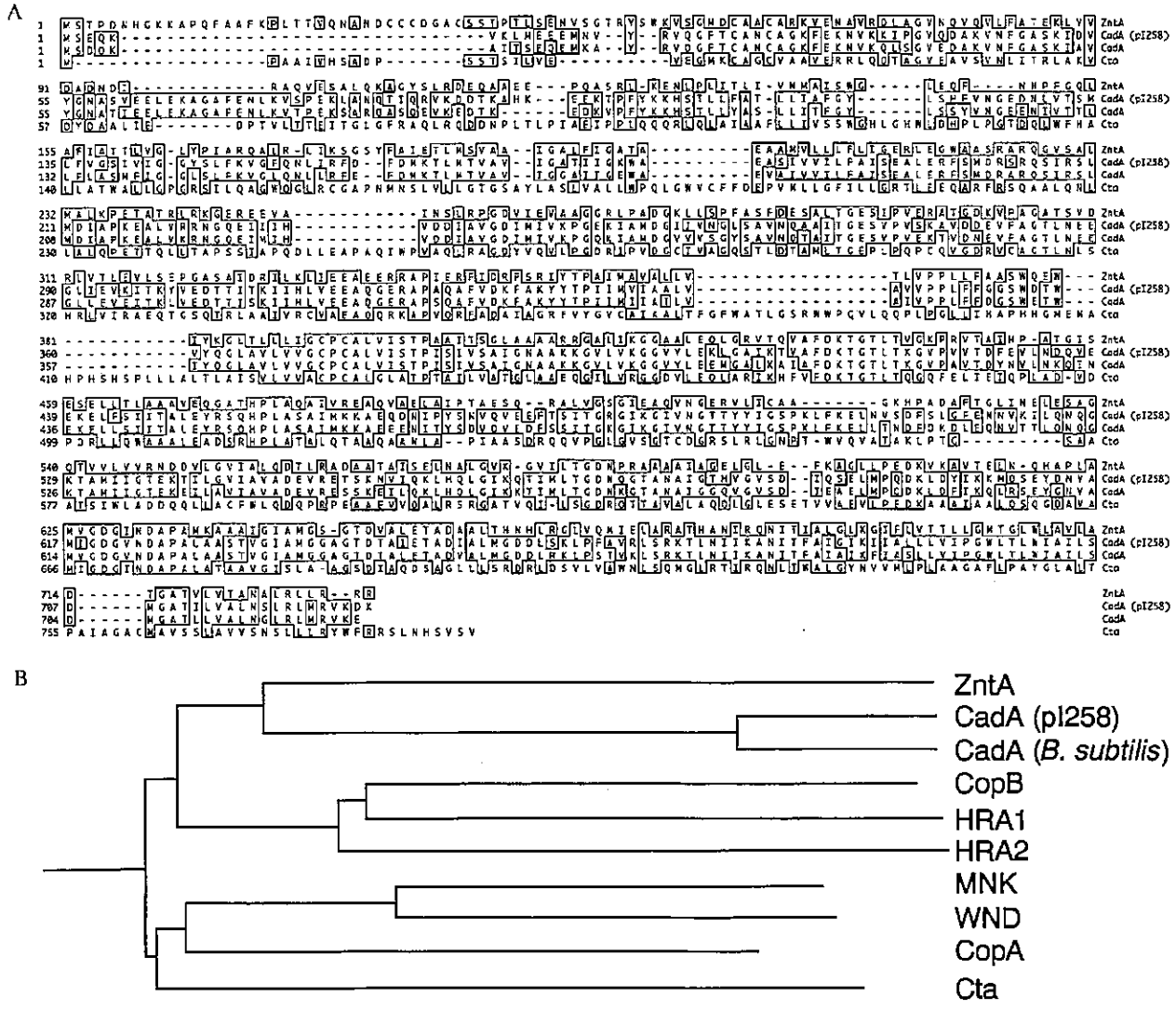

FIG. 1. Multiple alignment and phylogenetic relationship of the amino acid sequences of soft metal translocating P-type ATPases. (*A*) Alignment of the deduced amino acid sequence of ZntA (22); CadA (pI258) (14); CadA, *Bacillus firmus* (16); and CtaA, *Synechococcus* 7942 (18). Identical residues are boxed. (*B*) The dendogram was made with MEGALIGN (DNAstar). The branch lengths in the phylogenetic tree are proportional to the number of amino acid substitutions separating each pair. Proteins: ZntA (22), CadA (pI258) (14); CadA, *B. firmus* (16); CopB, *Enterococcus hirae* (17); HRA1, *E. coli* (21); HRA2, *E. coli* (21); Menkes protein (MNK) (19); Wilson protein (WND) (20); CopA, *Enterococcus hirae* (17); and CtaA, *Synechococcus* 7942 (18).

Table 1. Minimal inhibitory concentrations of metal salts

Metal salt	Apparent MIC, mM		
	W3110	RW3110	RW3110 (pCGR2)
$ZnSO_4$	2	0.5	2
$Cd(OAc)_2$	1.5	0.03	0.8
$CuCl_2$	4	4	4
$NiCl_2$	4	4	4
$CoCl_2$	1.6	1.6	1.6
$HgCl_2$	0.03	0.03	0.03
$AgNO_2$	0.6	0.6	0.6
$NaAsO_2$	5	5	5
Potassium antimonyl tartrate	1.2	1.2	1.2

Cells were grown overnight and streaked onto LB plates containing varying concentrations of metal salts. Growth was monitored after 24 hr at 37°C. The apparent minimal inhibitory concentration (MIC) is the concentration at which no colonies were observed.

quantified in a liquid scintillation counter. A blank value, obtained by filtering 0.1 ml of assay mixture without membrane vesicles, was subtracted from all points. To determine the initial rates, 1- and 2-min time points were used.

Other Methods. Protein sequence alignment and construction of a phylogenetic tree were executed with MEGALIGN from DNAstar (Madison, WI). Protein content was estimated by a modified procedure of Lowry *et al.* (29).

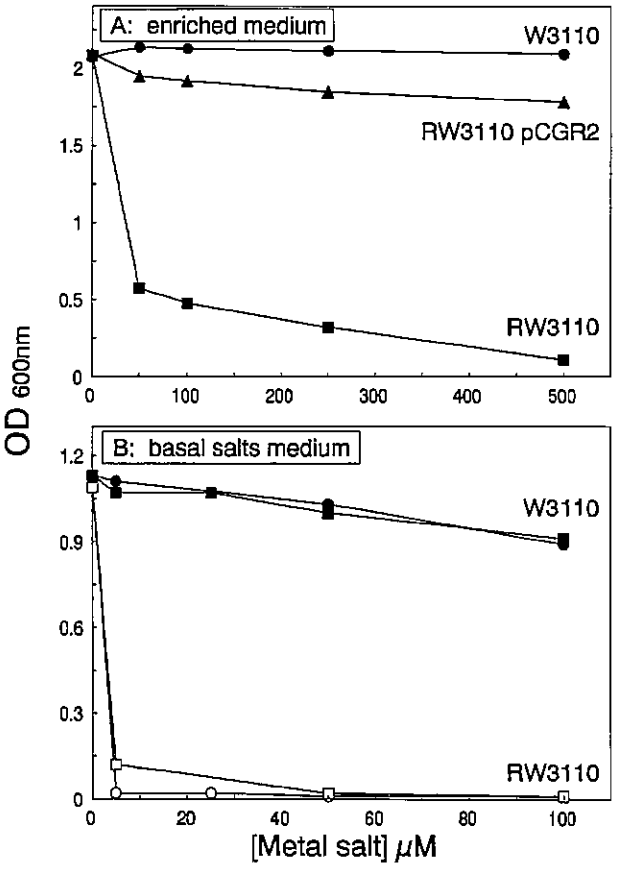

FIG. 2. Cadmium and zinc ion resistance. Metal ion resistance was assayed in cells of *E. coli* grown either in LB medium (*A*) or a basal salts medium (*B*) with the indicated concentrations of metal ion salts for 24 hr at 37°C with shaking, following which turbidity at 600 nm was measured. (*A*) Cadmium sensitivity in strain W3110 (wild type) (●), RW3110 (*zntA::kan*) (■), or RW3110 pCGR2 (*zntA*) (▲). (*B*) ■, Zinc sensitivity in W3110; □, zinc sensitivity in RW3110; ●, cadmium sensitivity in W3110; ○, cadmium sensitivity in RW3110.

RESULTS

The *zntA* Gene Confers Zinc and Cadmium Tolerance. CadA, a Cd(II)-translocating P-type ATPase, is encoded by the *cadA* gene of plasmid pI258 from *Staphylococcus aureus* (14). The closest homolog to CadA is the putative product of *o732* (Fig. 1*A*), one of several potential soft metal-translocating P-type ATPases identified in the *E. coli* genome (21, 22). These enzymes form a subfamily of the cation-translocating P-type ATPases that transport soft metals, including Cu(I), Ag(I), and Cd(II) (Fig. 1*B*) (11–13). Even though the *o732* gene product is closely related to CadA, the two proteins have only 35% identical residues. In particular, the *o732* gene product has an N-terminal extension containing an additional cysteine triplet that may be of possible functional significance. Thus it would not be unexpected for the chromosomally encoded *o732* and the plasmid-encoded *cadA* gene products to have different physiological activities.

The function of *o732* was investigated by disruption of the gene. The gene was cloned into plasmid pGEM-T by PCR, creating plasmid pCGR2. A kanamycin cassette was inserted into *o732*, and the chromosomal *o732* of the *recD* strain JCB499 was replaced with the disrupted gene by homologous recombination. The disruption was transduced into strain W3110, producing strain RW3110. The growth of the two strains was compared on solid LB medium containing various metal ion salts (Table 1). The two strains showed the same growth in the presence of copper, nickel, cobalt, mercury,

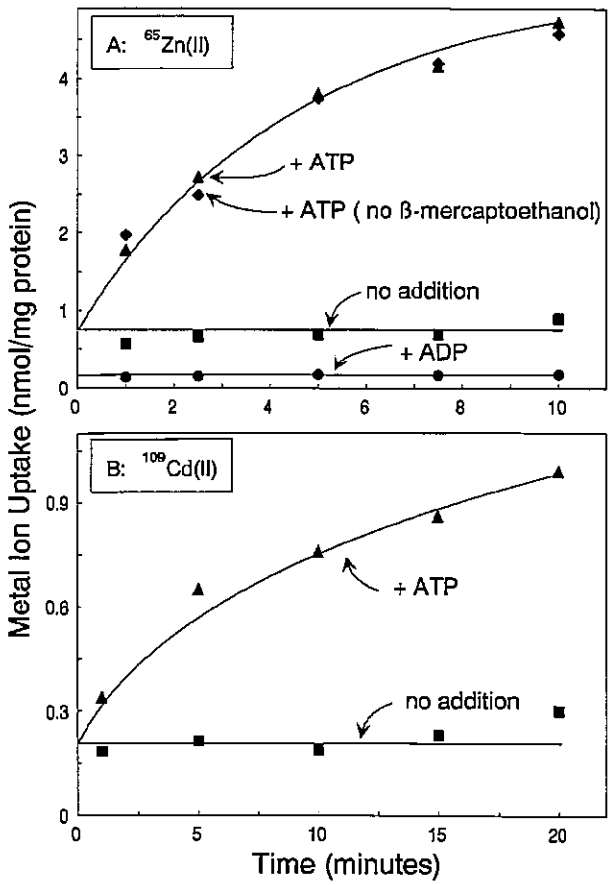

FIG. 3. ATP-dependent uptake of (*A*) ^{65}Zn(II) and (*B*) ^{109}Cd(II) in everted membrane vesicles of *E. coli*. Vesicles were prepared from cells of strain W3110 induced with 1 mM $ZnSO_4$. Transport was assayed with 10 μM $^{65}ZnSO_4$ or ^{109}Cd acetate. Additions: (■), no energy source; (▲), 5 mM MgATP; (●), 5 mM MgADP; (◆), 5 mM MgATP but 2-mercaptoethanol was omitted from the assay buffer.

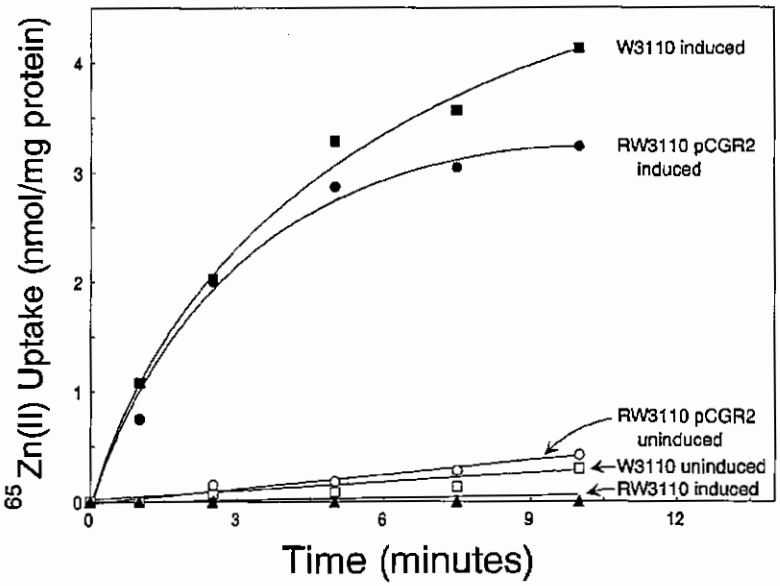

FIG. 4. Inducibility of ATP-dependent $^{65}Zn(II)$ transport in everted membrane vesicles and complementation by *zntA*. Vesicles were prepared from zinc-induced (filled symbols) or uninduced (open symbols) cells of *E. coli* W3110 (squares) or RW3110 (triangles) or RW3110 pCG2 (circles). The values at each time were corrected for nonspecific binding by subtraction of the nonenergy-dependent values.

silver, arsenic, and antimony. In contrast, RW3110 exhibited a 50-fold increase in sensitivity to cadmium and a 4-fold increase in zinc sensitivity. The cadmium sensitivity of the mutant in liquid LB medium could be complemented by pCGR2, demonstrating that the disruption was responsible for the phenotype (Fig. 2*A*). The response to both zinc and cadmium was more dramatic in a basal salts medium (Fig. 2*B*). The reason for the greater sensitivity to zinc in a basal medium compared with an enriched medium is not known, but complexation of zinc with components of LB medium is a possibility. Zinc resistance could also be restored by pCGR2 (data not shown). Whereas this work was in progress Poole and coworkers (30) reported the isolation of a zinc-sensitive strain by random transposon mutagenesis, designating the disrupted gene *zntA*. They showed the sequence of *zntA* to be identical to *o732*. The gene name *zntA* is used hereafter.

ATP-Dependent Accumulation of $^{65}Zn(II)$ and $^{109}Cd(II)$ in Everted Membrane Vesicles of *E. coli*. Everted membrane vesicles were prepared from cells of *E. coli* strain W3110. In the absence of a source of energy or in the presence of MgADP no time-dependent uptake of $^{65}Zn(II)$ (Fig. 3*A*) or $^{109}Cd(II)$ (Fig. 3*B*) was observed. Addition of MgATP produced time-dependent accumulation of both metals in the wild type. In subsequent experiments transport was corrected for nonenergy-dependent binding. Although 2-mercaptoethanol was included in most assays, it apparently was not required (Fig. 3*A*).

Properties of ZntA-Catalyzed Zinc Transport in E. coli. To demonstrate that transport requires expression of the *zntA*

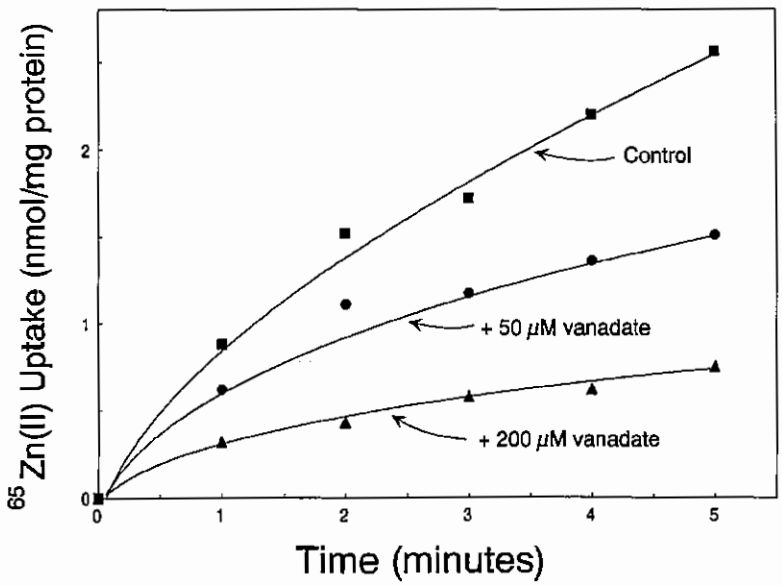

FIG. 5. Vanadate inhibition of $^{65}Zn(II)$ transport. Transport was assayed in vesicles prepared from zinc-induced cells of *E. coli* W3110 in the presence of the following concentrations of sodium orthovanadate: ■, none; ●, 50 μM; ▲, 200 μM. 5 mM MgATP was present as an energy source. The values at each time were corrected for nonspecific binding by subtraction of the non-energy-dependent values.

gene, accumulation of $^{65}Zn(II)$ was compared in everted membrane vesicles from cells of the wild-type strain W3110, the mutant *zntA::kan* strain RW3110, and the mutant complemented with a *zntA* gene on plasmid pCGR2 (Fig. 4). In uninduced cells only a basal level of activity was observed. Induction with 1 mM $ZnSO_4$ for 1 hr resulted in a dramatic increase in transport activity. Vesicles of the mutant exhibited no accumulation of $^{65}Zn(II)$, but the transport defect was complemented by the *zntA* gene on a plasmid. Zinc transport exhibited saturability, with an apparent K_m for Zn(II) of 9 μM (data not shown). From its sequence, ZntA would be predicted to be a P-type ATPase. This class of enzyme is sensitive to inhibition by vanadate. Consistent with this assumption, transport of $^{65}Zn(II)$ was inhibited by vanadate, with approximately 50% inhibition with 50 μM sodium orthovanadate (Fig. 5).

DISCUSSION

In this work we demonstrate that *o732*, a potential gene in the *E. coli* genome, is responsible for specific resistance to zinc and cadmium in *E. coli*. While this work was in progress Poole and coworkers (30) isolated by transposon mutagenesis a Zn(II)/Cd(II)-sensitive mutant of *E. coli*, designating the mutated gene *zntA*. The sequence of *zntA* was identical to that of *o732*, and we have adopted that gene name. *zntA* was predicted to code for a soft metal-translocating P-type ATPase (13). Our data clearly establish that ZntA catalyzes ATP-dependent zinc transport in everted membrane vesicles of *E. coli*. Because everted membranes have an orientation opposite to that of the inner membrane of intact cells, accumulation in vesicles is the equivalent of extrusion from cells (28).

Although the closest homolog of ZntA is CadA, the two proteins share only 35% identity. The physiological role of the plasmid-encoded CadA is clearly cadmium resistance. On the other hand, we would predict that ZntA has a dual role physiologically. Whereas it would confer cadmium resistance in the rare event that cells of *E. coli* would be exposed to cadmium, a more routine function would be in zinc homeostasis. Consistent with that idea, the pump appears to transport $^{65}Zn(II)$ approximately 4-fold more effectively than $^{109}Cd(II)$ (Fig. 3).

Total zinc in *E. coli* has been estimated as a nominal concentration of 0.6 mM if it were all free (31). In mammalian cells this value has been estimated at 0.2 mM and free intracellular Zn(II) at 20 μM (6). However, free intracellular zinc concentrations are difficult to measure. ZntA-mediated zinc transport in everted membrane vesicles exhibits a K_m in the range of 10^{-5} M zinc. *A priori*, it is likely that a K_m in the range of the free intracellular concentration would provide the greatest ability to respond to changes in cytosolic zinc. Although wild-type *E. coli* can tolerate zinc concentrations in the millimolar range, a strain with a disruption in *zntA* is sensitive to micromolar concentrations. These results suggest that free intracellular zinc may be in the range of 10^{-5} M.

Zinc transport was induced by growth in zinc. The *cadA* gene is regulated by the CadC repressor (32), a member of the ArsR family of metalloregulatory proteins (33). However, no genes for homologs of CadC or ArsR are located near *zntA*, and there are no nearby unknown reading frames encoding potential DNA-binding proteins (22). Expression of *zntA* from its own promoter on a plasmid remained inducible, even though the cloned sequence contained no additional reading frames. However, expression from that high copy number plasmid did not produce an increase in either resistance or transport. These results imply a requirement for a chromosomally encoded regulatory protein. Because expression from a high copy number plasmid would be likely to titrate out a small number of repressor proteins, positive regulation of *zntA* expression is a possibility. The sequence of the putative *zntA* promoter has similarities with the positively regulated promoter of the mercury resistance (*mer*) operon. Thus, whereas the mechanism of zinc regulation remains obscure, we would predict the existence of an activator.

Zinc is an essential metal required by all organisms. Zinc ions play both structural and catalytic roles in zinc-dependent proteins (1–3). In addition, transcription factors recently have been implied to control developmental changes by a *zinc-occupancy* switch that is dependent on the availability of Zn(II) inside cells (34). In spite of the many physiological roles of Zn(II), in high concentrations it is toxic. In *E. coli* ZntA can be considered as the outward half of a zinc homeostatic mechanism. The other half would be comprised of one or more zinc uptake systems. Other organisms have developed a variety of mechanisms to maintain intracellular zinc homeostasis. In both eukaryotes and prokaryotes Zn(II) hyperaccumulation is prevented by binding of metal to metallothioneins (4, 5). Secondary carriers confer zinc resistance by movement of zinc out of cells or into intracellular compartments. For example, the Czc antiporter of *Alcaligenes eutrophus* CH34 produces resistance to Co(II), Zn(II), and Cd(II) by extrusion in exchange for protons (9, 10). The mammalian transporters Znt-1 and Znt-2 mediate resistance to Zn(II) by translocation of Zn(II) across various membranes (6, 7). However, zinc resistance produced by a primary ATP-coupled pump has not been demonstrated to date in eukaryotes or other prokaryotes. Considering the inborn errors of copper homeostasis produced by mutations in the Wilson and Menkes genes, energy-dependent active efflux of zinc is likely to be important in humans, predictably by ZntA homologs.

We thank Drs. Robert Poole and Andy Morby for valuable discussions. Thanks are due Dr. James Bardwell for advice and for strain JCB499. This work was supported in part by United States Public Health Service Grants GM55425 (to B.P.R.), GM54102 (to B.M.), National Research Service Award GM18973 (to C.R.), a research award from Wayne State University (to B.M.), and a Wayne State University School of Medicine postdoctoral award (to C.R.).

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MDHS

Methods for the Determination of Hazardous Substances

Health and Safety Laboratory

HSE Health & Safety Executive

42/2

Nickel and inorganic compounds of nickel in air (except nickel carbonyl)

Laboratory method using flame atomic absorption spectrometry or electrothermal atomic absorption spectrometry

March 1996

INTRODUCTION

Note 1: *This method updates and replaces MDHS 42.[1] The principal changes which have been made are (i) to describe additional dissolution techniques that are effective for nickel-containing dusts that are difficult to take into solution, (ii) to recommend the use of filters that are soluble using the dissolution techniques described, and (iii) to describe the use of electrothermal atomic absorption spectrometry for the analysis of sample solutions with a low nickel concentration.*

Occurrence, properties and uses

1 Occurrence, properties and uses of nickel and its inorganic compounds are fully covered in HSE Guidance Note EH 60.[2]

Effects on health

2 The health effects of nickel and its inorganic compounds are summarised in HSE Guidance Note EH 60[2] and are fully covered in HSE Toxicity Review TR 19.[3]

Health and safety precautions

3 HSE leaflet MS(A)14[4] summarises the risks involved in working with nickel and what can be done to control them. Prevention and control of exposure, emergency procedures and health surveillance are described more fully in HSE Guidance Note EH 60.[2]

Exposure limits

4 Inorganic nickel compounds can be conveniently classified into two distinct groups according to their solubility in water, and use was made of this when setting occupational exposure limits for nickel and its inorganic compounds. The following table classifies some of the more commercially important nickel compounds on the basis of their solubility:

Water-soluble nickel compounds

	Solubility in water (18-20°C),[5] in grams litre^{-1}
nickel chloride $NiCl_2$	642
nickel nitrate $NiNO_3$	485
nickel sulphate $NiSO_4$	275
nickel sulphamate $Ni(SO_3NH_2)_2$	No data available
nickel cyanide complex	No data available

Water-insoluble nickel compounds

nickel carbonate $NiCO_3$	0.093[6]
nickel hydroxide $Ni(OH)_2$	0.013
nickel monoxide NiO	0.001
nickel sulphide NiS	0.004
nickel subsulphide Ni_3S_2	No data available

5 Schedule 1 of the Control of Substances Hazardous to Health (COSHH) Regulations[7] specifies three maximum exposure limits (MELs) for nickel and its inorganic compounds. These long-term exposure limits, 8-hour time-weighted average reference period, are also published in Table 2 of HSE Guidance Note EH 40,[8] and are reproduced below. The criteria on which the limits were based are documented in the 1993 edition of HSE Guidance Note EH 64.[9]

Nickel	0.5 mg m^{-3}
Nickel, inorganic compounds (as Ni)	
Soluble compounds	0.1 mg m^{-3}
Insoluble compounds	0.5 mg m^{-3}

A soluble nickel compound is defined (for this purpose) as any single nickel compound or complex which has a solubility greater than 10% by weight in water at 20°C.

Analytical methods

6 This is not a 'reference' method in the strict analytical sense of the word. There are frequently several alternative methods available for the determination of a particular analyte. With the exception of a few cases, where an exposure limit is linked to a specific method (eg rubber fume or asbestos), the use of methods not included in the MDHS series is acceptable provided that they have been shown to have the accuracy and reliability appropriate to the application.

7 This method has been validated[10] to demonstrate that it complies with the *General requirements for the performance of procedures for the measurement of chemical agents in workplace atmospheres* described by the Comité Européen de Normalization (CEN) in European Standard EN 482[11] (see paragraphs A1.7, A2.6, A3.6, A4.6 and A5.6). If an alternative method is used, it is necessary to demonstrate that it also meets these performance requirements.

Requirements of the COSHH Regulations

8 *The Control of Substances Hazardous to Health (COSHH) Regulations*[7] require that employers make an assessment of the health risk created by work involving substances hazardous to health, and to prevent or control exposure to such substances. The COSHH Regulations also include a requirement that persons who may be exposed to substances hazardous to health receive suitable and sufficient information, instruction and training. Employers must ensure that their responsibilities under the COSHH Regulations are fulfilled before allowing employees to undertake any procedure described in this method. Guidance is given in the Approved Codes of Practice for the Control of Substances Hazardous to Health, the *General COSHH ACOP*, and the Control of Carcinogenic Substances, the *Carcinogens ACOP*, which are included in a single publication with the COSHH Regulations.[12]

SCOPE

Applicability

9 This MDHS describes procedures for determination of the concentration of nickel and inorganic compounds of nickel in workplace air using either flame atomic absorption spectrometry or electrothermal atomic absorption spectrometry. It is applicable to the determination of water-soluble nickel compounds, insoluble nickel compounds and the majority of nickel-containing materials in industrial use or occurring in workplace air. The method is suitable for sampling times in the range 15 minutes to 8 hours.

Note 2: *HSE Guidance Note EH 42[13] advises employers about how they should conduct investigations into the nature, extent and control of exposure to substances hazardous to health which are present in workplace air. The objective of air monitoring is usually to determine worker exposure, and therefore the procedures described in this method are for personal sampling in the breathing zone. The method may be used for background or fixed location sampling, but it should be recognised that, due to aerodynamic effects, samplers designed for personal sampling do not necessarily exhibit the same collection characteristics when used for other purposes.*

METHOD PERFORMANCE

Effectiveness of sample dissolution procedures

10 A number of sample dissolution procedures have been described in this method (see paragraphs 61 to 64). Their effectiveness has been tested[10] on a range of nickel-containing materials in industrial use or occurring in workplace air, and this is reported in the relevant appendix.

Detection limits

11 The qualitative and quantitative detection limits for nickel, defined as three times and ten times the standard deviation of a blank determination, have been determined[10] separately for each of the sample dissolution procedures described in this method (see Appendices A1 to A5). The mean qualitative and quantitative detection limits were 0.016 μg ml^{-1} and 0.054 μg ml^{-1} respectively for flame atomic absorption spectrometry; and 0.9 ng ml^{-1} and 3.0 ng ml^{-1} for electrothermal atomic absorption spectrometry. For an air sample volume of 30 litres and a sample solution volume of 10 ml this corresponds to nickel in air concentrations of 6 μg m^{-3} and 18 μg m^{-3} for flame atomic absorption spectrometry; and 0.3 μg m^{-3} and 1.0 μg m^{-3} for electrothermal atomic absorption spectrometry.

Overall uncertainty

12 The bias of the analytical method and the component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), have been determined[10] separately for each of the sample dissolution procedures described in this method (see Appendices A1 to A5).

13 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] separately for each of the sample dissolution procedures described in this method (see Appendices A1 to A5). In all instances it was within the specification prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

Interferences

14 The analysis is based on atomic absorption spectrometry using an air-acetylene flame at a wavelength of 232.0 nm, where only minimal interferences have been found. Some transition metals have been reported[14] to interfere with the determination of nickel. The two worst cases of iron and chromium were both investigated[10] and found to be insignificant. No interferences specific to the determination of nickel are documented for electrothermal atomic absorption spectrometry.

PRINCIPLE

15 A measured volume of air is drawn through a filter mounted in an inhalable dust sampler. The sample is then taken into solution according to one of the five methods described in Appendices A1 to A5. The resultant solution is analysed for nickel by aspirating into the oxidising air-acetylene flame of an atomic absorption spectrometer. Absorbance measurements are made at 232.0 nm with background correction. For accurate determination when the concentration of nickel in the solution is low, the analysis may be repeated using electrothermal atomic absorption spectrometry. Aliquots of the sample solution are injected onto a solid, pyrolytic graphite platform mounted in a pyrolytically-coated graphite tube, and after drying and ashing stages the sample is atomised electrothermally. Absorbance measurements are made at 232.0 nm with background correction.

REAGENTS

16 During the analysis, use only reagents of recognised analytical grade. Use only distilled or de-ionised water, or water of equal purity (paragraph 17). Do not pipette by mouth.

Water

17 Water complying with the requirements of BS 3978[15] grade 2 water (electrical conductivity less than 0.1 mS m^{-1} and resistivity greater than 0.01 MΩ.m at 25°C).

Ammonium citrate leach solution, 1.7% (m/v) $(NH_4)_2HC_6H_5O_7$ and 0.5% (m/v) $C_6H_8O_7.H_2O$

18 Weigh 17 g of di-ammonium hydrogen citrate, $(NH_4)_2HC_6H_5O_7$, and 5 g of citric acid monohydrate, $C_6H_8O_7.H_2O$, into a 500 ml beaker. Add 250 ml water (paragraph 17) and swirl to dissolve. Quantitatively transfer the solution into a 1000 ml volumetric flask, dilute to the mark with water, stopper and mix thoroughly. Check, and if necessary, adjust the pH of the solution to 4.4 with ammonia or citric acid.

Note 3: *This leach solution is required only for the procedure described in Appendix A1.*

Nitric acid (HNO_3), concentrated, ρ about 1.42 g ml^{-1}, 69% (m/m) to 71% (m/m)

19 The nickel concentration of the acid shall be less than 0.005 µg ml^{-1}.

WARNING *- Concentrated nitric acid is corrosive and oxidising, and nitric acid fumes are irritant. Avoid exposure by contact with the skin or eyes, or by inhalation of fumes. Personal protection (eg gloves, face shield or safety spectacles etc) should be used when working with concentrated or diluted nitric acid, and sample dissolution with nitric acid should be carried out in a fume cupboard.*

Nitric acid, diluted 1 + 1

20 Carefully add 500 ml of concentrated nitric acid (paragraph 19) to 450 ml of water (paragraph 17) in a 2 litre beaker. Swirl to mix, allow to cool and quantitatively transfer to a 1 litre volumetric flask. Dilute to the mark with water, stopper and mix thoroughly.

Note 4: *1 + 1 nitric acid is required only for the procedure described in Appendix A2 and for preparation of the stock standard nickel solution in paragraph 26.*

Nitric acid, diluted 1 + 3

21 Carefully add 250 ml of concentrated nitric acid (paragraph 19) to 600 ml of water (paragraph 17) in a 2 litre beaker. Swirl to mix, allow to cool and quantitatively transfer to a 1 litre volumetric flask. Dilute to the mark with water, stopper and mix thoroughly.

Note 5: *1 + 3 nitric acid is required only for the procedure described in Appendix A5.*

Nitric acid, diluted 1 + 9

22 Add approximately 800 ml of water (paragraph 17) to a 1 litre volumetric flask. Carefully add 100 ml of concentrated nitric acid (paragraph 19) to the flask and swirl to mix. Allow to cool, dilute to the mark with water, stopper and mix thoroughly.

Perchloric acid ($HClO_4$), ρ about 1.67 g ml^{-1}, approximately 70% (m/m)

23 The nickel concentration of the acid shall be less than 0.002 µg ml^{-1}.

Note 6: *Perchloric acid is required only for the procedure described in Appendix A3.*

WARNING *- Perchloric acid is corrosive and oxidising, and its fumes are irritant. Avoid exposure by contact with the skin or eyes, or by inhalation of fumes. Personal protection (eg gloves, face shield or safety spectacles etc) should be used when working with concentreted or diluted perchloric acid, and sample dissolution with perchloric acid should be carried out in a fume cupboard with a scrubber unit specially designed for use with perchloric acid.*

Potassium hydrogen sulphate ($KHSO_4$)

24 The nickel content of the salt shall be less than 0.01 µg g^{-1}.

Note 7: *Potassium hydrogen sulphate is required only for the procedure described in Appendix A5.*

Stock standard nickel solution, 1000 µg ml^{-1} of nickel

25 Use a commercially available standard solution at a concentration of 1000 µg ml^{-1} of nickel. Observe the manufacturer's expiry date or recommended shelf life.

Alternatively prepare a stock nickel standard solution by the following procedure:

26 Accurately weigh 1.000 g of nickel metal, 99.9% Ni (m/m), into a 100 ml beaker, add 20 ml of 1 + 1 nitric acid (paragraph 20), cover with a watch glass and heat on a hotplate (paragraph 40) in a fume cupboard until the solid is completely dissolved. Remove the beaker from the hotplate, allow to cool, quantitatively transfer the solution into a 1 litre volumetric flask, dilute to the mark with water (paragraph 17), stopper and mix thoroughly.

Note 8: *Nickel standard solution prepared according to the instructions in paragraph 26 may be stored in a polypropylene bottle (paragraph 35) for a period of one year without deterioration.*

WARNING *- Nickel and nickel compounds have been assigned various risk phrases in the Approved Supply List[16] for the Chemicals (Hazard Information and Packaging for Supply) Regulations 1994[17] (the 'CHIP 2' Regulations). Care should be taken when working with nickel metal and solutions containing nickel.*

Working standard nickel solution, 1.00 µg ml^{-1} of nickel

27 Accurately pipette 100 µl of stock nickel standard solution (paragraph 25 or 26) into a 100 ml volumetric flask. Add 1 ml of concentrated nitric acid (paragraph 19), dilute to the mark with water (paragraph 17), stopper and mix thoroughly. Prepare this solution fresh daily.

Laboratory detergent solution

28 A laboratory grade detergent suitable for cleaning of samplers and labware, diluted with water (paragraph 17) according to the manufacturer's instructions.

SAMPLING EQUIPMENT

Samplers for collection of the inhalable fraction of the airborne particles

29 Samplers, with protective covers, for collection of the inhalable fraction of the airborne particles, as defined in European Standard EN 481.[18] Inhalable dust samplers suitable for personal sampling are described in MDHS 14.[19]

Note 9: *In general, the collection characteristics of inhalable samplers can be such that particulate material collected on the filter is the inhalable fraction of the airborne particles, and any deposited on the internal surfaces of the sampler is not of interest. However, some samplers are designed such that airborne particles which pass through the entry orifice(s) constitute the inhalable fraction, in which case any particulate material deposited on the internal surfaces of the sampler is part of the sample. Certain samplers of this type incorporate an internal filter cassette which may be removed from the sampler to enable this material to be easily recovered. Refer to the manufacturer's instructions to ascertain what constitutes the inhalable fraction of the sample.*

Note 10: *Samplers manufactured in non-conducting material have electrostatic properties which may influence representative sampling. Electrostatic influences should be reduced, where possible, by using samplers manufactured from conducting material.*

Filters

30 Filters, of a diameter suitable for use in the samplers (paragraph 29), with a retentivity of not less than 99.5% for particles with a 0.3 µm diffusion diameter. The use of filters that are soluble using the sample preparation procedures described in Appendices A2 to A5 is recommended, and mixed cellulose ester membrane filters of 0.8 µm mean pore diameter are considered to be most suitable.

The nickel content shall be less than 0.001 µg per filter.

Note 11: *Glass fibre or other filters which do not dissolve using the sample preparation procedure described may be used, but extra care needs to be taken to ensure quantitative transfer of sample solutions to volumetric flasks (see Appendices A1 to A5).*

Sampling pumps

31 Sampling pumps, complying with the provisions of draft European Standard prEN 1232,[20] with an adjustable flow rate, incorporating a flowmeter or a flow fault indicator, capable of maintaining the appropriate flow rate (see paragraph 48) to within ±5% of the nominal value throughout the sampling period (see paragraph 49), and capable of being worn by persons without impeding normal work activity. The pumps shall give a pulsation-free flow (if necessary, a pulsation damper shall be incorporated between the sampler and the pump, as near to the pump as possible). Flow-stabilised pumps may be required to maintain the flow rate within the specified limits.

Flowmeter

32 Flowmeter, portable, capable of measuring the appropriate flow rate (see paragraph 48) to within ±5%, and calibrated against a primary standard.

Note 12: *The flowmeter incorporated in the pump may be used provided that it has adequate sensitivity, that it has been calibrated against a primary standard with a loaded filter in line, and that it is read in a vertical orientation if it is of the supported float type. However, it is important to ensure that there are no leaks in the sampling train between the sampler and the flowmeter, since in this event a flowmeter in the pump or elsewhere in line will give an erroneous flow rate.*

Note 13: *A soap bubble flowmeter may be used as a primary standard, provided its accuracy is traceable to national standards (see Appendix B).*

Ancillary equipment

33 Flexible plastic tubing, of a diameter suitable for making a leakproof connection from the sampler to the

sampling pump; belts or harnesses to which the sampling pump can conveniently be fixed, unless the pump is sufficiently small to fit in the worker's pocket; flat-tipped tweezers for loading and unloading the filters into samplers; and filter transport cassettes or similar, if required (see paragraph 57), to transport samples to the laboratory.

LABORATORY APPARATUS

Glassware, made of borosilicate glass

34 A selection of laboratory glassware, including: beakers; watch glasses; measuring cylinders; test tubes; and one-mark volumetric flasks, class A, complying with the requirements of BS 1792.[21]

Note 14: *It is recommended that a set of glassware is reserved for the analysis of nickel by this method (see paragraph 68).*

Polypropylene bottle

35 A polypropylene bottle, with leakproof screw cap, for storage of stock standard solution (paragraph 26), cleaned before use by soaking in 1 + 9 nitric acid (paragraph 22) for at least 24 hours and then rinsing thoroughly with water (paragraph 17). A bottle made of an alternative plastic may be used provided that it is suitable for the intended use.

Disposable gloves

36 Disposable gloves, impermeable, to avoid the possibility of contamination from the hands and to protect them from contact with toxic and corrosive substances. PVC gloves are suitable.

Piston operated volumetric apparatus

37 A set of adjustable micropipettes, complying with the requirements of BS 7653-1 to BS 7653-4,[22-25] for the preparation of working standard nickel solution (paragraph 27) and calibration solutions (paragraphs A1.14, A1.15, A2.12, A2.13, A3.12, A4.14 and A5.16), and dilution of sample solutions (paragraphs 76, 86 and A5.15). A suitable set might include micropipettes covering the ranges 10 µl to 100 µl, 100 µl to 1000 µl and 1000 µl to 5000 µl. Dispensers for dispensing acid.

Filter paper

38 A hardened, ashless, cellulose (paper) filter of medium filtering speed and retentivity.

Suction filtration apparatus

39 Suction filtration apparatus, for filtration of the citrate leach solution used in the sample dissolution procedure for water-soluble nickel (see Appendix A1). Suitable apparatus comprises of a water-operated or electrically driven vacuum pump, connected to a conical flask fitted with a filter funnel/support assembly (see Figure 1).

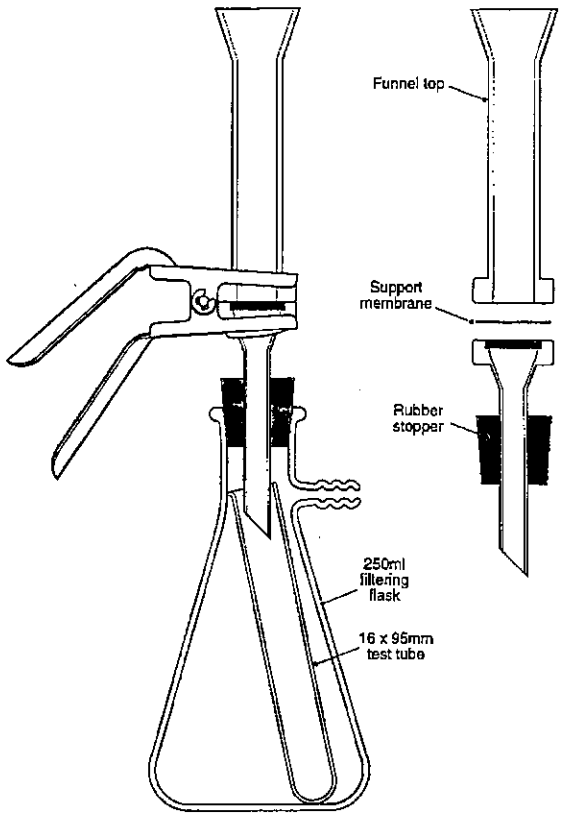

Figure 1 *Suction filtration apparatus*

Cellulose ester membrane filters, of a diameter suitable for use with the apparatus, are also required.

Note 15: *Alternative suction filtration apparatus is available which permits simultaneous vacuum filtration of multiple samples.*

Note 16: *The filtration apparatus is only required for the procedure described in Appendix A1.*

Hotplate

40 A thermostatically controlled hotplate, capable of maintaining the required surface temperature.

Microwave digestion system

41 A commercial, closed vessel microwave digestion system, designed for laboratory use, with power output regulation, equipped with a turntable of sample vessels able to withstand pressures in excess of 100 psi, and fitted with a pressure control system.

Note 17: *The microwave digestion system and vessels are only required for the procedure described in Appendix A4.*

Porcelain crucibles

42 Fused porcelain crucibles, low form, 25 ml capacity, with matching lids.

Meker burner

43 Natural gas burner, Meker pattern, Amal major.

Muffle furnace

44 Electric muffle furnace capable of maintaining a temperature of 650°C.

Note 18: *Porcelain crucibles, Meker burner and muffle furnace are only required for the procedure described in Appendix A5.*

Atomic absorption spectrometer

45 An atomic absorption spectrometer, fitted with an air-acetylene burner, supplied with compressed air and acetylene, and equipped with a nickel hollow cathode lamp. If electrothermal atomic absorption spectrometry is to be carried out, the atomic absorption spectrometer shall be capable of carrying out simultaneous background correction at 232.0 nm, either by using a continuum source such as a deuterium lamp to measure non-specific attenuation, or by using Zeeman or Smith-Hieftje background correction systems.

Electrothermal atomiser

46 An electrothermal atomiser, fitted with a solid, pyrolytic graphite platform mounted in a pyrolytically-coated graphite tube, supplied with argon as a purge gas, and equipped with an autosampler capable of injecting microlitre volumes onto the platform.

Note 19: *Some manufacturers of atomic absorption spectrometers use an alternative design of electrothermal atomiser to achieve a constant temperature environment during atomisation, and some use aerosol deposition as a means of sample introduction. The use of such accessories is acceptable, but the method performance may be different from that described in paragraphs 11 to 13, A1.4 to A1.7 and A2.3 to A2.6.*

Disposable autosampler cups

47 Disposable polystyrene autosampler cups for use in the autosampler used with the electrothermal atomiser. Soak in 1 + 9 nitric acid (paragraph 22) before use.

Note 20: *Disposable polystyrene autosampler cups are also useful for containing solutions to be pipetted in microlitre quantities.*

SAMPLING

Sampling procedure

48 Use the samplers (paragraph 29) at the design flow rate, so that they exhibit the required collection characteristics.

49 Select a suitable sampling time, such that the filter does not become overloaded with aerosol. (An 8-hour time weighted average concentration may be derived from the results for two or more consecutive samples, as described in Guidance Note EH 42.[13])

Preparation of sampling equipment

Perform the following in an area where nickel contamination is known to be low.

50 Clean the samplers (paragraph 29) before use. Disassemble the samplers, soak in laboratory detergent solution (paragraph 28), rinse thoroughly with water (paragraph 17), wipe with absorptive tissue and allow to dry thoroughly before reassembly. Alternatively, use a laboratory washing machine.

51 Load the filters (paragraph 30) into clean, dry samplers (see paragraph 50) using clean, flat-tipped tweezers (paragraph 33). Connect each loaded sampler to a sampling pump (paragraph 31) using plastic tubing (paragraph 33), ensuring that no leaks can occur. Switch on the pump, attach the calibrated flowmeter (paragraph 32) to the sampler so that it measures the flow through the sampler inlet orifice, and set the appropriate flow rate (see paragraph 48) with an accuracy of ±5%. Remove the flowmeter and allow the pump to operate for an appropriate period to enable it to warm up and the flow rate to stabilise (follow the manufacturer's recommendations). Then discard the used filter and load a new one into the sampler for collection of the sample. Finally, attach the calibrated flowmeter again, readjust the flow rate to the appropriate value with an accuracy of ±5%, switch off the pump and seal the sampler with its protective cover to prevent contamination with nickel during transport to the sampling position.

Collection of samples

52 Fix the sampler to the lapel of the worker, in the breathing zone and as close to the mouth and nose as practicable. Then, either place the sampling pump in a convenient pocket or attach it to the worker in a manner that causes minimum inconvenience, eg to a belt (paragraph 33) around the waist. When ready to begin sampling, remove the protective cover from the sampler and switch on the pump. Record the time at the start of the sampling period, and if the pump is equipped with an elapsed time indicator, set this to zero.

53 Since it is possible for a filter to become clogged, monitor the performance of the sampler frequently, a minimum of once per hour. Measure the flow rate with an accuracy of ±5% using the calibrated flowmeter (paragraph 32) and record the measured value. Terminate sampling and consider the sample to be invalid if the flow rate is not maintained to within ±5% of the nominal value throughout the sampling period.

Note 21: *Regular observation of the flow fault indicator is an acceptable means of ensuring that the flow rate of flow-stabilised sampling pumps is maintained satisfactorily, provided that the flow fault indicator indicates malfunction when the flow rate is outside ±5% of the nominal value.*

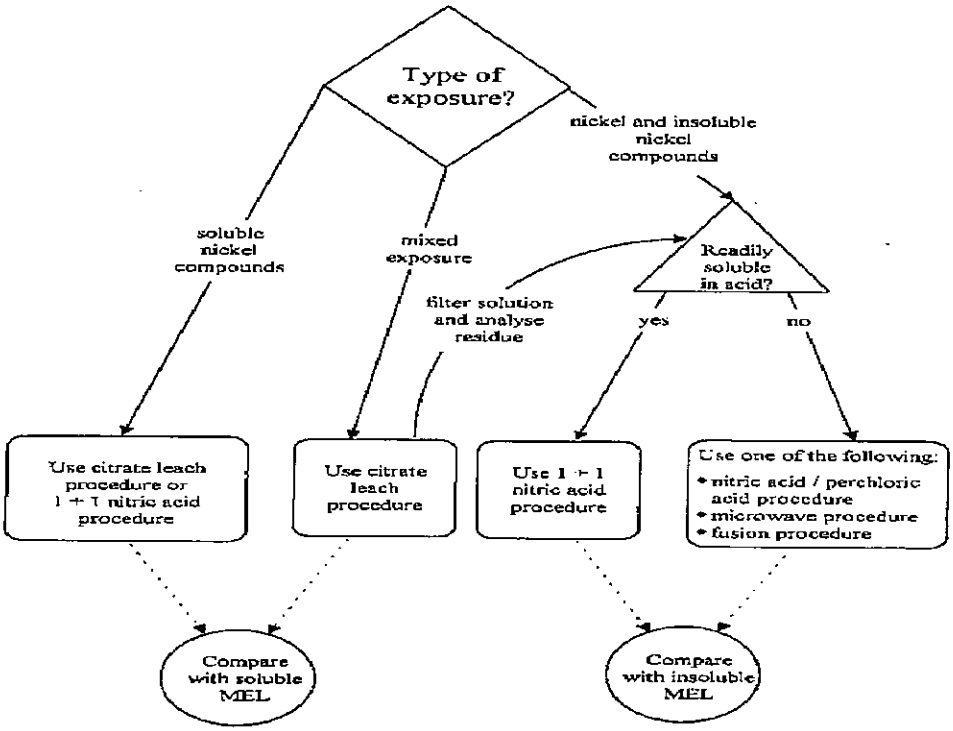

Figure 2 *Schematic diagram for the selection of suitable sample dissolution procedure(s) according to the nature of the nickel-containing material present in the test atmosphere*

54 At the end of the sampling period (see paragraph 49), measure the flow rate with an accuracy of ±5% using the calibrated flowmeter (paragraph 32), switch off the sampling pump, and record the flow rate and the time. Also observe the reading on the elapsed time indicator, if fitted, and consider the sample to be invalid if the reading on the elapsed time indicator and the timed interval between switching on and switching off the sampling pump do not agree to within ±5%, since this may suggest that the sampling pump has not been operating throughout the sampling period. Reseal the sampler with its protective cover and disconnect it from the sampling pump.

55 Carefully record the sample identity and all relevant sampling data (see Appendix D). Calculate the mean flow rate by averaging the flow rate measurements taken throughout the sampling period and calculate the volume of air sampled, in litres, by multiplying the flow rate in litres per minute by the sampling time, in minutes.

56 With each batch of ten samples, submit for analysis two unused filters from the same lot of filters used for sample collection. Subject these blank filters to exactly the same handling procedure as the samples, but draw no air through them.

Transportation

Perform the following in an area where nickel contamination is known to be low.

57 For samplers which collect the inhalable fraction of airborne particles on the filter (see note 9), remove the filter from each sampler using clean flat-tipped tweezers (paragraph 33), place in a labelled filter transport cassette (paragraph 33) and close with a lid.

58 For samplers which have an internal filter cassette (see note 9), remove the filter cassette from each sampler, fasten with the transport clip supplied by the manufacturer, and label appropriately.

59 For samplers designed such that airborne particles which pass through the entry orifice(s) constitute the inhalable fraction but which do not have an internal filter cassette (see note 9), and for samplers of the disposable cassette type, transport the samples to the laboratory in the samplers in which they were collected.

60 Transport the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) to the laboratory in a container which has been designed to prevent damage to samples in transit and which has been labelled to assure proper handling.

ANALYSIS

Wear disposable gloves (paragraph 36) during analysis to reduce the possibility of contamination and to protect the hands from corrosive and oxidising reagents.

Selection of sample dissolution procedure

61 Select a suitable sample dissolution procedure(s) according to the nature of the nickel-containing material present in the test atmosphere (see Figure 2).

Water-soluble nickel compounds

62 If it is known that no insoluble nickel compounds are in use in the workplace (see paragraph 4), and that none are produced in the processes carried out, use either the citrate leach procedure described in Appendix A1 or the 1 + 1 nitric acid dissolution procedure described in Appendix A2, and compare results with the MEL for soluble nickel compounds.

Note 22: *The procedure described in Appendix A2 is not specific for soluble nickel compounds. However, it may be used as an alternative to the procedure described in Appendix A1, in the circumstances described above, if this is more convenient.*

Nickel metal and water-insoluble nickel compounds

63 If it is known that no soluble nickel compounds are in use in the workplace (see paragraph 4), and that none are produced in the processes carried out, select one of the procedures described in Appendices A2 to A5, and compare results with the MEL for insoluble nickel compounds. Take into consideration the nature of the nickel-containing material present in the test atmosphere and the availability of laboratory apparatus. In many instances the nature of the matrix will be such that nickel is readily soluble in acid, in which case use the 1 + 1 nitric acid dissolution procedure described in Appendix A2. However, if nickel is in a more difficult matrix, eg refractory dusts or stainless steel welding fume, use one of the more vigorous dissolution procedures.

Mixed exposure to water-soluble and water-insoluble nickel compounds

64 If water-soluble and water-insoluble nickel compounds could be present in the test atmosphere, use the citrate leach procedure described in Appendix A1 to determine water-soluble nickel, and compare results with the MEL for soluble nickel compounds. Then select one of the procedures described in Appendices A2 to A5 (see paragraph 63), analyse the residue for water-insoluble nickel compounds, and compare results with the MEL for insoluble nickel compounds.

Cleaning of glassware

65 Before use, clean all glassware (paragraph 34) to remove any residual grease or chemicals. Firstly soak overnight in laboratory detergent solution (paragraph 28) and then rinse thoroughly with water (paragraph 17). Alternatively, use a laboratory washing machine.

66 After initial cleaning (see paragraph 65), clean all beakers used in the sample dissolution procedures (see paragraphs A2.8 to A2.11 and A3.8 to A3.11) with hot nitric acid. Fill to one third capacity with concentrated nitric acid (paragraph 19), cover with a watch glass, heat to approximately 150°C on the hotplate (paragraph 40) in a fume cupboard for 1 hour, allow to cool, and then rinse thoroughly with water (paragraph 17).

67 After initial cleaning (see paragraph 65), clean all glassware other than beakers used in the sample dissolution procedure by soaking in 1 + 9 nitric acid (paragraph 22) for at least 24 hours and then rinsing thoroughly with water (paragraph 17).

68 Glassware which has been previously subjected to the cleaning procedure described in paragraphs 65 to 67, and which has been reserved for determination of nickel by this method, can be adequately cleaned by rinsing thoroughly with 1 + 9 nitric acid (paragraph 22) and then with water (paragraph 17).

Preparation of sample and blank solutions

69 Refer to the relevant appendix and prepare the sample and blank solutions using the selected sample dissolution procedure (see paragraphs 61 to 64).

ANALYSIS BY FLAME ATOMIC ABSORPTION SPECTROMETRY

Preparation of calibration solutions

70 Prepare matrix-matched calibration solutions. Refer to the appendix relevant to the selected sample dissolution procedure (see paragraphs 61 to 64).

Atomic absorption measurements

71 Set up the atomic absorption spectrometer (paragraph 45) to determine nickel at a wavelength of 232.0 nm using an oxidising air-acetylene flame. Follow the manufacturer's recommendations for specific operating parameters, and use background correction. The sensitivity, defined as the concentration required to produce a signal of 1% absorbance or 0.0044 absorbance units, is about 0.07 $\mu g\ ml^{-1}$ of nickel.

72 Adjust the spectrometer zero while aspirating the blank calibration solution (paragraph 70). Repeat this procedure regularly throughout the analysis and readjust the zero if the baseline drifts.

73 Aspirate the calibration solutions (paragraph 70) into the flame in order of increasing concentration and make absorption measurements for each solution. For instruments controlled by a microprocessor or personal computer, generate a calibration for nickel by carrying out a linear regression. For instruments without this capability, prepare a calibration graph by plotting the absorbance of the calibration solutions versus the nickel concentration.

74 Aspirate the sample and blank solutions (paragraph 69) into the flame and make absorption measurements for each solution. For instruments controlled by a microprocessor or personal computer, use the calibration function to determine the concentration of nickel in the sample and blank solutions and obtain a direct read-out of the results in $\mu g\ ml^{-1}$ of nickel. For instruments without this capability, determine the concentration of nickel in $\mu g\ ml^{-1}$ from the calibration graph (paragraph 73).

75 Aspirate a mid-range calibration solution into the flame after each five to ten sample solutions and make an absorption measurement. If this indicates that the sensitivity has changed by more than ±5%, take one of the following appropriate corrective measures: either use the available software facilities of the microprocessor or personal computer to correct for the sensitivity change (reslope facility); or suspend analysis, recalibrate the spectrometer as described in paragraph 73; and in either case reanalyse the solutions which were analysed during the period in which the sensitivity change occurred.

76 If high concentrations of nickel are found, dilute the sample solutions to bring the concentration within the calibration range, and repeat the analysis. Make all dilutions so that the final matrix is consistent with the dissolution procedure used. Record the dilution factor.

77 Calculate the mean nickel concentration of the blank solutions.

78 If the concentration of nickel in the sample solutions is less than 0.5 μg ml^{-1} consider repeating the analysis using electrothermal atomic absorption spectrometry (see paragraphs 79 to 87) since this technique gives more precise measurements at low concentrations.

Note 23: *Electrothermal atomic absorption spectrometry is required only for the procedures described in Appendices A1 and A2*

ANALYSIS BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

Note 24: *Nickel is present at a low level in the environment and it is essential that strict standards of cleanliness are observed to avoid contamination of labware. This is particularly important when carrying out electrothermal atomic absorption spectrometry since the technique exhibits a very low detection limit. Ensure that all glassware is cleaned thoroughly before use in accordance with paragraphs 65 to 68, and that autosampler cups (paragraph 47) are stored in 1 + 9 nitric acid (paragraph 22) until required.*

Note 25: *The use of electrothermal atomic absorption spectrometry is only necessary where exposure to water-soluble nickel compounds needs to be determined. Flame atomic absorption spectrometry has been shown to meet the CEN requirements for measurement of water-insoluble nickel compounds, which have a higher maximum exposure limit.*

Preparation of working calibration solutions

79 Prepare a matrix-matched working calibration solution. Refer to the appendix relevant to the selected sample dissolution procedure (see paragraphs 61 to 64).

80 Prepare a matrix-matched working calibration blank solution. Refer to the appendix relevant to the selected sample dissolution procedure (see paragraphs 61 to 64).

Atomic absorption measurements

81 Set up the atomic absorption spectrometer (paragraph 45) and electrothermal atomiser (paragraph 46) to determine nickel at a wavelength of 232.0 nm using background correction. Follow the manufacturer's recommendations for specific operating parameters.

Note 26: *The operating parameters for electrothermal atomic absorption spectrometry vary considerably between different instruments, much more so than for flame atomic absorption spectrometry. A Perkin-Elmer 5100PC atomic absorption spectrometer with Zeeman HGA-600 graphite furnace module and AS-60 autosampler was used in the validation of this method,[10] and the operating parameters used are given in Appendix C. The characteristic mass for nickel, defined as the number of picograms required to give 0.0044 absorbance-seconds, was determined to be 12 pg for this analytical system. This is equivalent to a sample solution concentration of 0.60 ng ml^{-1} of nickel for a 20 μl sample solution injection volume.*

82 Program the autosampler to prepare calibration solutions in situ on a pyrolytic graphite platform mounted in the pyrolytically-coated graphite tube of the electrothermal atomiser. Prepare at least six calibration solutions to cover the range 0 ng ml^{-1} to 50 ng ml^{-1} using the working calibration solution (paragraph 79) and the working calibration blank solution (paragraph 80). See Table 2 for typical autosampler injection volumes.

Note 27: *The procedure described above may be varied to accommodate the use of electrothermal atomisers of alternative design (see note 19).*

Note 28: *Calibration solutions may be prepared in volumetric flasks as an alternative to preparation in situ using the autosampler.*

Note 29: *Sample test solutions should be diluted (see paragraph 86) before analysis by electrothermal atomic absorption spectrometry (see paragraph 78) if results obtained by flame atomic absorption spectrometry indicate that the nickel concentration is above the upper limit of the calibration range for electrothermal atomic absorption spectrometry (see paragraph 82).*

83 Set-up the analytical sequence in the microprocessor or personal computer. Specify an appropriate number of replicate analyses for each solution, and insert a calibration blank solution and a mid-range calibration solution after each five to ten sample solutions to monitor for baseline drift and sensitivity change respectively.

84 Place the working calibration solution (paragraph 79), the working calibration blank solution (paragraph 80), and the sample and blank solutions (paragraph 69) in separate acid-washed autosampler cups (see note 24) and position as appropriate in the autosampler carousel. Analyse the calibration, sample and blank solutions, using the microprocessor or personal computer software to generate a calibration and obtain a direct read-out of sample and blank results in ng ml^{-1} of nickel.

85 If significant baseline drift is observed during the course of the analysis, or if the sensitivity changes by more than ±5%, take one of the following appropriate corrective measures: either use the available software facilities of the microprocessor or personal computer to correct for the sensitivity change (reslope facility); or suspend analysis and recalibrate the spectrometer as described in paragraph 84. In either case reanalyse the solutions which were analysed during the period in which the sensitivity change occurred.

86 If concentrations of nickel above the upper limit of the calibration range are found, dilute the sample solutions to bring them within the calibration range, and repeat the analysis. Make all dilutions so that the final matrix is consistent with the dissolution procedure used. Record the dilution factor.

87 Calculate the mean nickel concentration of the blank solutions.

QUALITY CONTROL MEASURES

88 Analytical quality requirements, guidance on the establishment of a quality assurance programme and details of internal quality control and external quality assessment schemes are fully described in MDHS 71.[26]

89 If nickel analysis is performed frequently it is recommended that internal quality control is performed. In such instances, prepare quality control filters by spiking a large batch of filters with microlitre volumes of a solution of known nickel concentration. Analyse a random selection of at least 20 filters, each along with a different analytical batch, and calculate the mean value and standard deviation of the readings. Assuming that the distribution of these values is Gaussian, construct a Shewhart chart with warning and action limits at ±2SD and ±3SD respectively. Subsequently, analyse a quality control filter with each analytical batch and plot the result on the Shewhart chart. Compare the internal quality control result with the target value and take appropriate action if the warning or action limits are exceeded, as recommended in MDHS 71.[26] Take care to ensure that the quality control filters are stored under conditions which ensure maximum stability.

90 It is strongly recommended that all laboratories undertaking the determination of toxic elements in workplace air should participate in an external quality assessment scheme such as HSE's Workplace Analysis Scheme for Proficiency (WASP). Details of WASP are given in MDHS 71.[26] However, at present the WASP scheme does not encompass nickel.

CALCULATIONS

Volume of air sample

91 Calculate the mean flow rate during the sampling period by averaging the flow rate measurements taken at the start and end of the sampling period. Then calculate the volume, in litres, of the air sample by multiplying the mean flow rate, in litres per minute, by the sampling time, in minutes.

Concentration of nickel in air

92 Calculate the concentration of nickel in air, $\rho(Ni)$, in milligrams per cubic metre (mg m^{-3}), using the equation:

$$\rho(Ni) = \frac{[\rho(Ni)_1.V_1.DF_1 - \rho(Ni)_0.V_0.DF_0]}{V}$$

where $\rho(Ni)_0$ is the mean concentration, in µg ml^{-1}, of nickel in the blank solutions (see paragraphs 77 and 87);

$\rho(Ni)_1$ is the concentration, in µg ml^{-1}, of nickel in the sample solution (see paragraphs 74 and 84);

V is the volume, in litres, of the air sample (see paragraph 91);

V_0 is the volume, in ml, of the blank solutions, ie 10 ml or 50 ml (see paragraph 69);

V_1 is the volume, in ml, of the sample solution, ie 10 ml or 50 ml (see paragraph 69);

DF_0 is the dilution factor for the blank solutions, ie 1;

DF_1 is the dilution factor for the sample solutions (see paragraphs 76 and 86).

Note 30: *For low concentrations of nickel in air determined by electrothermal atomic absorption spectrometry, calculate results in micrograms per cubic metre by using solution concentrations in ng ml^{-1} in the above equetion.*

TEST REPORT

93 Appendix D gives recommendations for information to be included in the test report.

APPENDIX A1 Citrate leach procedure for dissolution of water-soluble nickel compounds

SCOPE

A1.1 This appendix describes a dissolution procedure for water-soluble nickel compounds using an ammonium citrate leach solution.

METHOD PERFORMANCE

Effectiveness of sample dissolution procedure

A1.2 A citrate leach procedure for the determination of water-soluble nickel compounds was tested by NiPERA[27] in an interlaboratory test programme, and was found[10] to be fully effective.

Note 31: *The citrate leach procedure was considered to be preferable to a simple water leach because (i) buffering the pH ensures reproducibility by minimising the effects on nickel solubility caused by foreign hydrolysable salts; (ii) the complexing ability of the citrate ion towards higher valency elements, eg Fe(III), prevents unwanted precipitation of hydrolytic products; and (iii) nickel forms relatively weak complexes with citrate, so solubility in citrate solution is the same as that in water.*

A1.3 The procedure described in this method has been modified somewhat to avoid what were considered to be unnecessary steps. The revised procedure was compared with that described by NiPERA using one of the dusts from the interlaboratory test programme, and was found[10] to be equally effective.

Note 32: *The changes that were made to the procedure described by NiPERA were to omit the use of methanol to remove any hydrophobic organic material and to make the dust wettable. Methanol destroys the mixed cellulose ester membrane filters recommended in this procedure. The ammonium citrate leach solution was oxidised by NiPERA using perchloric acid; this step was omitted as it was found that the ammonium citrate did not interfere with the determination of nickel by either flame or electrothermal atomic absorption spectrometry.*

Detection limits

A1.4 The qualitative and quantitative detection limits as determined[10] for this procedure were 0.012 µg ml^{-1} and 0.040 µg ml^{-1} respectively for flame atomic absorption spectrometry; and 0.96 ng ml^{-1} and 3.2 ng ml^{-1} for electrothermal atomic absorption spectrometry. For an air sample volume of 30 litres and a sample solution volume of 10 ml this corresponds to nickel in air concentrations of 4 µg m^{-3} and 13 µg m^{-3} respectively for flame atomic absorption spectrometry; and 0.3 µg m^{-3} and 1.1 µg m^{-3} respectively for electrothermal atomic absorption spectrometry.

Overall uncertainty

A1.5 Laboratory experiments[10] indicate that the analytical method does not exhibit significant bias. The mean analytical recovery for 100 spiked filters in the range 0.6 µg to 192 µg of nickel was determined to be 99.4 ± 3.5% using flame atomic absorption spectrometry; and the mean analytical recovery for 60 spiked filters in the range 0.05 µg to 3 µg of nickel was determined to be 100.9 ± 3.8% using electrothermal atomic absorption spectrometry.

A1.6 The component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), was determined[10] to be less than 6% for samples in the range 0.6 µg to 2.4 µg and less than 3% for samples in the range 3 µg to 192 µg using flame atomic absorption spectrometry; and less than 7% for samples at a level of 0.05 µg and less than 4% for samples in the range 0.1 µg to 3 µg using electrothermal atomic absorption spectrometry.

A1.7 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] to be less than 26% for samples in the range 0.6 µg to 2.4 µg and less than 13% for samples in the range 3 µg to 192 µg using flame atomic absorption spectrometry; and less than 23% for samples at a level of 0.05 µg and less than 17% for samples in the range 0.1 µg to 3 µg using electrothermal atomic absorption spectrometry. This assumes that the coefficient of variation of the method that arises from inter-specimen sampler variability, CV (inter), is negligible and that the coefficient of variation of the method that arises from pump flow rate variability, CV (flow), is limited to 5%. The overall uncertainty is therefore within the specifications prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

PRINCIPLE

A1.8 The soluble nickel compounds are leached from the sample filter by immersing the filter in a solution of ammonium citrate. The resultant leach solution is filtered through a membrane filter and diluted to 10 ml for subsequent analysis by either flame or electrothermal atomic absorption spectrometry.

PREPARATION OF SAMPLE AND BLANK SOLUTIONS

A1.9 Open the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) and transfer each filter into an individual, labelled 50 ml beaker using clean flat-tipped tweezers (paragraph 33). Follow the same procedure for the blank filters (paragraph 56).

A1.10 If the sampler used was of a type in which airborne particles deposited on the internal surfaces of the filter cassette or sampler form part of the sample (see note 9),

wash any particulate material adhering to the internal surfaces into the beaker using the ammonium citrate solution used to leach the sample filters (see paragraph A1.11).

A1.11 Add 5 ml ammonium citrate solution (paragraph 18) to each beaker, cover with a watch glass, and allow to leach for 60 minutes. Ensure that the sample filters are fully immersed throughout the leach period.

A1.12 Filter each leach solution through a cellulose ester membrane filter using suction filtration apparatus (paragraph 39), collecting the filtrate in an individual, labelled test tube (see Figure 1). Rinse the sample filter and beaker with three 1 ml aliquots of ammonium citrate solution, allowing the solution to completely drain from the filter funnel between washings. Quantitatively transfer the filtrate to a 10 ml volumetric flask, rinsing out the test tube with a further 1 ml of ammonium citrate solution. Finally, dilute to the mark with ammonium citrate solution, stopper and mix thoroughly.

A1.13 If applicable (see paragraph 64), retain the sample filter and the secondary filter (the membrane filter used for filtration of the leach solution) for subsequent analysis for water-insoluble nickel compounds.

PREPARATION OF CALIBRATION SOLUTIONS

Flame atomic absorption spectrometry

A1.14 Prepare at least six calibration solutions to cover the range 0 μg ml^{-1} to 5 μg ml^{-1} of nickel. Accurately pipette the appropriate volumes of stock standard nickel solution (paragraph 25 or 26) into separate, labelled 100 ml volumetric flasks, dilute to the mark with ammonium citrate solution (paragraph 18), stopper and mix thoroughly. Prepare these solutions fresh weekly.

Electrothermal atomic absorption spectrometry

A1.15 Prepare a working calibration solution at a concentration of 50 ng ml^{-1} of nickel. Accurately pipette 0.5 ml of working standard nickel solution (paragraph 27) into a 100 ml volumetric flask. Dilute to the mark with ammonium citrate solution (paragraph 18), stopper and mix thoroughly. Prepare this solution fresh daily.

A1.16 Use the ammonium citrate solution as a working calibration blank solution.

ANALYSIS

Flame atomic absorption spectrometry

A1.17 Proceed with the analysis as described in paragraphs 71 to 78.

Electrothermal atomic absorption spectrometry

A1.18 Proceed with the analysis as described in paragraphs 81 to 87.

APPENDIX A2 1 + 1 Nitric acid sample dissolution procedure for nickel and water-insoluble nickel compounds

SCOPE

A2.1 This appendix describes a dissolution procedure for nickel and water-insoluble nickel compounds using 1 + 1 nitric acid on a hotplate.

METHOD PERFORMANCE

Effectiveness of sample dissolution procedure

A2.2 This sample dissolution procedure has been tested[10] on a range of nickel-containing materials in industrial use or occurring in workplace air and has been found to be effective for nickel metal, nickel oxide (black), nickel oxide (green), nickel hydroxide, nickel phosphide and nickel sulphide.

Detection limits

A2.3 The qualitative and quantitative detection limits as determined[10] for this procedure were 0.024 μg ml^{-1} and 0.080 μg ml^{-1} respectively for flame atomic absorption spectrometry; and 0.87 ng ml^{-1} and 2.9 ng ml^{-1} for electrothermal atomic absorption spectrometry. For an air sample volume of 30 litres and a sample solution volume of 10 ml this corresponds to nickel in air concentrations of 8 μg m^{-3} and 27 μg m^{-3} respectively for flame atomic absorption spectrometry; and 0.3 μg m^{-3} and 1.0 μg m^{-3} respectively for electrothermal atomic absorption spectrometry.

Overall uncertainty

A2.4 Laboratory experiments[10] indicate that the analytical method does not exhibit significant bias. The mean analytical recovery for 170 spiked filters in the range 0.6 μg to 960 μg of nickel was determined to be 102.4 ± 3.0% using flame atomic absorption spectrometry; and the mean analytical recovery for 60 spiked filters in the range 0.05 μg to 3.0 μg of nickel was determined to be 102.1 ± 1.1% using electrothermal atomic absorption spectrometry.

A2.5 The component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), was determined[10] to be less than 14% for samples at a level of 0.60 μg and less than 6% for samples in the range 2.4 μg to 960 μg using flame atomic absorption spectrometry; and less than 8% for samples in the range 0.05 μg to 3 μg using electrothermal atomic absorption spectrometry.

A2.6 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] to be less than 30% for samples in the range 0.6 μg to 2.4 μg and less than 19% for samples in the range 3 μg to 960 μg using flame atomic absorption spectrometry; and less than 23% for samples at a level of 0.05 μg and less than 19% for

samples in the range 0.10 µg to 3 µg using electrothermal atomic absorption spectrometry. This assumes that the coefficient of variation of the method that arises from inter-specimen sampler variability, CV (inter), is negligible and that the coefficient of variation of the method that arises from pump flow rate variability, CV (flow), is limited to 5%. The overall uncertainty is therefore within the specifications prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

PRINCIPLE

A2.7 The filter and collected sample are treated with 5 ml of 1 + 1 nitric acid and heated on a hotplate until about 1 ml of concentrated nitric acid solution remains. This is diluted to 10 ml for subsequent analysis for nickel by either flame or electrothermal atomic absorption spectrometry.

PREPARATION OF SAMPLE AND BLANK SOLUTIONS

A2.8 Open the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) and transfer each filter into an individual, labelled 50 ml beaker using clean flat-tipped tweezers (paragraph 33). Follow the same procedure for the blank filters (paragraph 56).

A2.9 If the sampler used was of a type in which airborne particles deposited on the internal surfaces of the filter cassette or sampler form part of the sample (see note 9), wash any particulate material adhering to the internal surfaces into the beaker using a minimum volume of water (paragraph 17).

A2.10 Add 5 ml of 1 + 1 nitric acid (paragraph 20) to each beaker, partially cover with a watch glass, and heat to approximately 150°C on a hotplate (paragraph 40) in a fume cupboard until the filter has dissolved and the solution has been reduced to approximately 1 ml. Remove each beaker from the hotplate and allow to cool.

A2.11 Carefully rinse the watch glass and the sides of each beaker with water (paragraph 17) and quantitatively transfer the solution to an individual, labelled 10 ml volumetric flask. If necessary, remove any undissolved particulate material by filtering through a cellulose (paper) filter (paragraph 38) which has been pre-washed with 1 + 9 nitric acid (paragraph 22) and then with water. Finally dilute to the mark with water, stopper and mix thoroughly.

PREPARATION OF CALIBRATION SOLUTIONS

Flame atomic absorption spectrometry

A2.12 Prepare at least six calibration solutions to cover the range 0 µg ml^{-1} to 5 µg ml^{-1} of nickel. Add 50 ml of water (paragraph 17) and 10 ml of concentrated nitric acid (paragraph 19) to separate, labelled 100 ml volumetric flasks. Accurately pipette the appropriate volume of stock standard nickel solution (paragraph 25 or 26) into each flask and swirl to mix. Allow to cool, dilute to the mark with water, stopper and mix thoroughly. Prepare these solutions fresh weekly.

Electrothermal atomic absorption spectrometry

A2.13 Prepare a working calibration solution at a concentration of 50 ng ml^{-1} of nickel. Add 50 ml of water (paragraph 17) and 10 ml of concentrated nitric acid (paragraph 19) to a labelled 100 ml volumetric flask. Accurately pipette 0.5 ml of working standard nickel solution (paragraph 27) into the flask and swirl to mix. Allow to cool, dilute to the mark with water, stopper and mix thoroughly. Prepare this solution fresh daily.

A2.14 Prepare a working calibration blank solution following the above procedure but omitting the 0.5 ml of working standard nickel solution (paragraph 27).

ANALYSIS

Flame atomic absorption spectrometry

A2.15 Proceed with the analysis as described in paragraphs 71 to 78.

Electrothermal atomic absorption spectrometry

A2.16 Proceed with the analysis as described in paragraphs 81 to 87.

APPENDIX A3 Nitric acid/perchloric acid sample dissolution procedure for nickel and water-insoluble nickel compounds

SCOPE

A3.1 This appendix describes a dissolution procedure for nickel and water-insoluble nickel compounds using nitric acid and perchloric acid on a hotplate. It is particularly useful in instances when nickel compounds present in the sample are not readily soluble in acid, and the 1 + 1 nitric acid procedure described in Appendix A2 could be ineffective.

METHOD PERFORMANCE

Effectiveness of sample dissolution procedure

A3.2 This sample dissolution procedure has been tested[10] on a range of nickel-containing materials in industrial use or occurring in workplace air and has been found to be effective for welding fumes and dusts from nickel smelting operations.

Detection limits

A3.3 The qualitative and quantitative detection limits as determined for this procedure were 0.008 μg ml^{-1} and 0.026 μg ml^{-1} respectively for flame atomic absorption spectrometry. For an air sample volume of 30 litres and a sample solution volume of 10 ml this corresponds to nickel in air concentrations of 3 μg m^{-3} and 9 μg m^{-3} respectively.

Overall uncertainty

A3.4 Laboratory experiments[10] indicate that the analytical method does not exhibit significant bias. The mean analytical recovery for 100 spiked filters in the range 3 μg to 960 μg of nickel was determined to be 101.5 ± 2.7% using flame atomic absorption spectrometry.

A3.5 The component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), was determined[10] to be less than 3% for samples in the range 3 μg to 960 μg using flame atomic absorption spectrometry.

A3.6 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] to be less than 17% for samples in the range 3 μg to 960 μg using flame atomic absorption spectrometry. This assumes that the coefficient of variation of the method that arises from inter-specimen sampler variability, CV (inter), is negligible and that the coefficient of variation of the method that arises from pump flow rate variability, CV (flow), is limited to 5%. The overall uncertainty is therefore within the specifications prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

PRINCIPLE

A3.7 The filter and collected sample are treated with 5 ml of nitric acid and 1 ml of perchloric acid and heated on a hotplate until about 1 ml of acid solution remains. This is diluted to 10 ml for subsequent analysis for nickel by flame atomic absorption spectrometry.

PREPARATION OF SAMPLE AND BLANK SOLUTIONS

A3.8 Open the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) and transfer each filter into an individual, labelled 50 ml beaker using clean flat-tipped tweezers (paragraph 33). Follow the same procedure for the blank filters (paragraph 56).

A3.9 If the sampler used was of a type in which airborne particles deposited on the internal surfaces of the filter cassette or sampler form part of the sample (see note 9), wash any particulate material adhering to the internal surfaces into the beaker using a minimum volume of water (paragraph 17).

A3.10 Add 5 ml of concentrated nitric acid (paragraph 19), cover the beaker and digest on a hotplate until the filter dissolves. Slide back the watch glasses so that the beakers are only partially covered, add 1 ml of perchloric acid (paragraph 23), and continue to heat until dense, white fumes of perchloric acid are evolved and until approximately 1 ml of acid remains. Remove each beaker from the hotplate and allow to cool.

A3.11 Carefully rinse the watch glass and the sides of each beaker with water (paragraph 17) and quantitatively transfer the solution to an individual, labelled 10 ml volumetric flask. If necessary, remove any undissolved particulate material by filtering through a cellulose (paper) filter (paragraph 38) which has been pre-washed with 1 + 9 nitric acid (paragraph 22) and then with water. Finally dilute to the mark with water, stopper and mix thoroughly.

PREPARATION OF CALIBRATION SOLUTIONS

Flame atomic absorption spectrometry

A3.12 Prepare at least six calibration solutions to cover the range 0 μg ml^{-1} to 5 μg ml^{-1} of nickel. Add 50 ml of water (paragraph 17) and 10 ml of perchloric acid (paragraph 23) to separate, labelled 100 ml volumetric flasks. Accurately pipette the appropriate volume of stock standard nickel solution (paragraph 25 or 26) into each flask and swirl to mix. Allow to cool, dilute to the mark with water, stopper and mix thoroughly. Prepare these solutions fresh weekly.

ANALYSIS

Flame atomic absorption spectrometry

A3.13 Proceed with the analysis as described in paragraphs 71 to 78.

APPENDIX A4 Microwave-assisted sample dissolution procedure for nickel and water-insoluble nickel compounds

SCOPE

A4.1 This appendix describes a microwave-assisted dissolution procedure for nickel and water-insoluble nickel compounds using nitric acid. It is particularly useful in instances when nickel compounds present in the sample are not readily soluble in acid, and the 1 + 1 nitric acid procedure described in Appendix A2 could be ineffective.

Note 33: *The procedure described is for use with lined sample vessels designed for carrying out microwave digestions at pressures up to 200 psi. These vessels consist of a chemically resistant inner liner and cover (usually made of Teflon), which contains and isolates the sample solution from a higher strength outer pressure vessel structure. Other types of sample vessel designed to operate at equivalent or higher pressures may be used.*

METHOD PERFORMANCE

Effectiveness of sample dissolution procedure

A4.2 This sample dissolution procedure has been tested[10] on a range of nickel-containing materials in industrial use or occurring in workplace air and has been found to be effective for welding fumes and dusts from nickel smelting operations.

Detection limits

A4.3 The qualitative and quantitative detection limits as determined[10] for this procedure were 0.024 μg ml^{-1} and 0.080 μg ml^{-1} respectively for flame atomic absorption spectrometry. For an air sample volume of 30 litres and a sample solution volume of 50 ml this corresponds to nickel in air concentrations of 0.04 mg m^{-3} and 0.13 mg m^{-3} respectively.

Overall uncertainty

A4.4 Laboratory experiments[10] indicate that the analytical method does not exhibit significant bias. The mean analytical recovery for 100 spiked filters in the range 3 μg to 960 μg of nickel was determined to be 101.8 ± 1.7% using flame atomic absorption spectrometry.

A4.5 The component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), was determined[10] to be less than 17% for samples in the range 3 μg to 12 μg and less than 3% for samples in the range 15 μg to 960 μg using flame atomic absorption spectrometry.

A4.6 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] to be less than 40% for samples at a level of 3 μg and less than 18% for samples in the range 12 μg to 960 μg using flame atomic absorption spectrometry. This assumes that the coefficient of variation of the method that arises from inter-specimen sampler variability, CV (inter), is negligible and that the coefficient of variation of the method that arises from pump flow rate variability, CV (flow), is limited to 5%. The overall uncertainty is therefore within the specifications prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

PRINCIPLE

A4.7 The filter and collected sample are digested with 5 ml of concentrated nitric acid in a closed vessel using a microwave digestion system. The resultant solution is diluted to 50 ml for subsequent analysis for nickel by flame atomic absorption spectrometry.

CLEANING OF TEFLON LINERS

A4.8 Clean the teflon liners of the sample vessels with concentrated nitric acid before use. Follow the procedure described in paragraphs A4.11 and A4.12 and then rinse the liners thoroughly with water (paragraph 17).

PREPARATION OF SAMPLE AND BLANK SOLUTIONS

A4.9 Open the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) and transfer each filter into the teflon liner of a labelled sample vessel using clean flat-tipped tweezers (paragraph 33). Follow the same procedure for the blank filters (paragraph 56).

A4.10 If the sampler used was of a type in which airborne particles deposited on the internal surfaces of the filter cassette or sampler form part of the sample (see note 9), wash any particulate material adhering to the internal surfaces into the teflon liner using a minimum volume of water (paragraph 17).

A4.11 Add 5 ml of concentrated nitric acid (paragraph 19) to each liner and cover with its lid. Then seal the sample vessels with their screw caps and place them, evenly distributed, in the turntable of the microwave digestion system (paragraph 41).

A4.12 Program the microwave digestion system to operate for 30 minutes at full power, using the pressure control system to prevent the pressure in the control vessel exceeding 100 psi. When the program has run, allow the vessels to cool and the pressure to return to <10 psi.

A4.13 Remove the turntable from the microwave digestion system and place in a fume cupboard. Carefully open each sample vessel, rinse the lid and sides of the liner with water (paragraph 17), and quantitatively transfer the solution to an individual, labelled 50 ml volumetric flask. If necessary, remove any undissolved particulate matter by filtering through a cellulose (paper) filter (paragraph 38) which has been pre-washed with 1 + 9 nitric acid (paragraph 22) and then with water. Finally dilute to the mark with water, stopper and mix thoroughly.

Note 34: *The procedure described is for a microwave digestion system with a nominal output power of 700 W with pressure control. If the system also has temperature control, this can also be utilised to control the sample dissolution conditions, but the method performance could be different from that given above.*

PREPARATION OF CALIBRATION SOLUTIONS

Flame atomic absorption spectrometry

A4.14 Prepare at least six calibration solutions to cover the range 0 μg ml^{-1} to 5 μg ml^{-1} of nickel. Add 50 ml of water (paragraph 17) and 10 ml of concentrated nitric acid (paragraph 19) to separate, labelled 100 ml volumetric flasks. Accurately pipette the appropriate volume of stock standard nickel solution (paragraph 25 or 26) into each flask and swirl to mix. Allow to cool, dilute to the mark with water, stopper and mix thoroughly. Prepare these solutions fresh weekly.

ANALYSIS

Flame atomic absorption spectrometry

A4.15 Proceed with the analysis as described in paragraphs 71 to 78.

APPENDIX A5 Potassium hydrogen sulphate fusion procedure for nickel and water-insoluble nickel compounds

SCOPE

A5.1 This appendix describes a fusion procedure for nickel and water-insoluble nickel compounds using potassium hydrogen sulphate. It is particularly useful in instances when nickel compounds present in the sample are not readily soluble in acid, and the dissolution procedure described in Appendices A2, A3 and A4 could be ineffective.

Note 35: The fusion procedure is only suitable for dissolution of samples collected for sampling times in the range 2 to 8 hours. For shorter sampling times, the overall uncertainty does not meet the specification prescribed by CEN[11] for measurements in the range 0.1 to 0.5 times the limit value, ie <50%.

METHOD PERFORMANCE

Effectiveness of sample dissolution procedure

A5.2 This sample dissolution procedure has been tested[10] on a range of nickel-containing materials in industrial use or occurring in workplace air and has been found to be effective for welding fumes and dusts from nickel smelting operations.

Detection limits

A5.3 The qualitative and quantitative detection limits as determined[10] for this procedure were 0.021 µg ml^{-1} and 0.071 µg ml^{-1} respectively for flame atomic absorption spectrometry. For an air sample volume of 30 litres and a sample dilution volume of 250 ml this corresponds to nickel in air concentrations of 0.17 mg m^{-3} and 0.6 mg m^{-3} respectively.

Overall uncertainty

A5.4 Laboratory experiments[10] indicate that the analytical method does not exhibit significant bias. The mean analytical recovery for 54 spiked filters in the range 12 µg to 960 µg of nickel was determined to be 100.6 ± 2.2% using flame atomic absorption spectrometry.

A5.5 The component of the coefficient of variation of the method that arises from analytical variability, CV(analysis), was determined[10] to be less than 6% for samples in the range 12 µg to 960 µg using flame atomic absorption spectrometry.

A5.6 The overall uncertainty of the method, as defined by CEN,[11] was estimated[10] to be less than 17% for samples in the range 12 µg to 960 µg using flame atomic absorption spectrometry. This assumes that the coefficient of variation of the method that arises from inter-specimen sampler variability, CV (inter), is negligible and that the coefficient of variation of the method that arises from pump flow rate variability, CV (flow), is limited to 5%. The overall uncertainty is therefore within the specifications prescribed by CEN[11] for measurements for comparison with limit values, ie <50% for measurements in the range 0.1 to 0.5 times the limit value and <30% for measurements in the range 0.5 to 2.0 times the limit value.

PRINCIPLE

A5.7 The filter and collected sample are placed in a fused porcelain crucible and wet-ashed with 0.5 ml concentrated nitric acid. 2.5 g of molten potassium hydrogen sulphate is then poured over the sample which is then covered with the crucible lid and placed in a muffle furnace at 650°C for 1 hour. The fused bead is dissolved in 10 ml of 1 + 3 nitric acid, made to 25 ml with water, and further diluted by a factor of ten for subsequent analysis for nickel by flame atomic absorption spectrometry.

PREPARATION OF SAMPLE AND BLANK SOLUTIONS

A5.8 Open the filter transport cassettes (see paragraph 57), sampler filter cassettes (see paragraph 58) or samplers (see paragraph 59) and transfer each filter into an individual, labelled 30 ml fused porcelain crucible (paragraph 42) using clean flat-tipped tweezers (paragraph 33). Follow the same procedure for the blank filters (paragraph 56).

A5.9 If the sampler used was of a type in which airborne particles deposited on the internal surfaces of the filter cassette or sampler form part of the sample (see note 9), wash any particulate material adhering to the internal surfaces into the crucible using a minimum volume of water (paragraph 17).

A5.10 Add 0.5 ml of concentrated nitric acid (paragraph 19) to each crucible and wet-ash the filter by gently heating over a Meker burner (paragraph 43) and slowly boiling off the nitric acid (care should be taken to avoid loss of sample during heating as the acid will spit if heated strongly).

A5.11 Weigh 2.5 g portions of potassium hydrogen sulphate (paragraph 24) into separate porcelain crucibles (paragraph 42) and heat over a Meker burner (paragraph 43) until molten. Then pour a portion of molten flux over each ashed sample (paragraph A5.10) and cover with a crucible lid.

A5.12 Place the covered crucibles in a muffle furnace (paragraph 44) at a temperature of 650°C. Remove the

crucibles after 1 hour and place on a heat-proof surface and allow to cool.

A5.13 Remove the crucible lids and gently tap each fused bead into an individual, labelled 50 ml beaker. Rinse each crucible with two 5 ml aliquots of 1 + 3 nitric acid (paragraph 21) and add the washings to the beaker. Cover the beakers with watch glasses and heat on a hotplate to dissolve the fused beads.

A5.14 Carefully rinse the watch glass and the sides of each beaker with water (paragraph 17) and quantitatively transfer each solution to an individual, labelled 25 ml volumetric flask. If necessary, remove any undissolved particulate material by filtering through a cellulose (paper) filter (paragraph 38) which has been pre-washed with 1 + 9 nitric acid (paragraph 22) and then with water. Finally dilute to the mark with water, stopper and mix thoroughly.

A5.15 Further dilute the sample solutions for analysis. Accurately pipette 1 ml of each sample solution (paragraph A5.14) into an individual, labelled 10 ml volumetric flask, dilute to the mark with water (paragraph 17), stopper and mix thoroughly.

PREPARATION OF CALIBRATION SOLUTIONS

Flame atomic absorption spectrometry

A5.16 Prepare at least six calibration solutions to cover the range 0 µg ml^{-1} to 5 µg ml^{-1} of nickel. Add 50 ml of water (paragraph 17) and 10 ml of 1 + 9 nitric acid (paragraph 22) to separate, labelled 100 ml volumetric flasks. Weigh out an appropriate number of 1 g portions of potassium hydrogen sulphate (paragraph 24), transfer a portion to each flask and swirl to dissolve. Accurately pipette the appropriate volume of stock standard nickel solution (paragraph 25 or 26) into each flask, dilute to the mark with water (paragraph 17), stopper and mix thoroughly. Prepare these solutions fresh weekly.

ANALYSIS

Flame atomic absorption spectrometry

A5.17 Proceed with the analysis as described in paragraphs 71 to 78.

APPENDIX B Primary standard for calibration of portable flowmeter

The primary standard should preferably be a flowmeter whose accuracy is traceable to national standards, used with careful attention to the conditions of the calibration certificate. A bubble flowmeter may be used. This is an arrangement whereby the pump under test draws a soap film up a calibrated tube. The passage of the film is accurately timed between two marks whose separation defines a known volume. A one litre burette can form a suitable tube. The volume between the marks can be checked by filling the burette with water (paragraph 17), allowing temperatures to stabilise, drawing off a known volume and weighing the water, making allowance for the dependence of volume on temperature. A suitable bubble solution can be made by mixing one part of concentrated washing-up liquid, two parts glycerol and four parts water. The burette must be thoroughly wetted with the solution and several attempts at drawing the film up the tube may be necessary before the tube is wet enough for this to be achieved consistently. (Traceability of the calibration will require checking of the clocks and use of certificated weights.)

APPENDIX C Typical operating parameters for determination of nickel by electrothermal atomic absorption spectrometry

Mode:	Peak area
Integration time:	10 seconds
Background correction:	Zeeman
Injection volumes:	20 µl of calibration, sample or blank solution

Table 1 *Typical temperature profile for determination of nickel using electrothermal atomic absorption spectrometry*

Step	*Ramp time (sec)*	*Hold time (sec)*	*Furnace temp (°C)*	*Argon flow (ml min^{-1})*	*Read*
1 Dry	1	50	120	300	
2 Ash	1	30	1400	300	
3 Cool down	1	15	20	300	
4 Atomise	0	10	2600	0	*
5 Clean	1	5	2700	300	

Table 2 *Typical autosampler injection volumes for the in-situ preparation of calibration, sample and blank solutions*

	Volume of working calibration solution (µl)	*Volume of working calibration blank solution (µl)*	*Volume of sample or blank solution (µl)*
0 ng ml^{-1} calibration solution	-	20	-
10 ng ml^{-1} calibration solution	4	16	-
20 ng ml^{-1} calibration solution	8	12	-
30 ng ml^{-1} calibration solution	12	8	-
40 ng ml^{-1} calibration solution	16	4	-
50 ng ml^{-1} calibration solution	20	-	-
Sample or blank solution	-	-	20
Sample solution dilution	-	(20 - x)	x

APPENDIX D Recommendations for the test report

It is recommended that the test report should include the following information:

a) a complete identification of the air sample, including the date of sampling, the place of sampling, and the identity of the individual whose breathing zone was sampled;

b) a reference to this MDHS and a description of any deviation from the procedures described;

c) the type and diameter of filter used;

d) the type of sampler used;

e) the type of sampling pump used;

f) the type of flowmeter used, the primary standard against which it was calibrated, and the range of flow rates for which the flowmeter was calibrated;

g) the time at the start and at the end of the sampling period, and the sampling time in minutes;

h) the volume of air sampled, in litres;

i) the name of the person who collected the sample;

j) the time-weighted average mass concentration of nickel found in the air sample, in milligrams per cubic metre;

k) the name of the analyst;

l) the date of the analysis.

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Chapter 6.10 Nickel

General description

Nickel (Ni) is a silvery-white, hard metal. Although it forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell *(1)*. Nickel compounds that are practically insoluble in water include carbonate, sulfides (the main forms being amorphous or crystalline monosulfide, NiS, and subsulfide Ni_3S_2) and oxides (NiO, Ni_2O_3). Water-insoluble nickel compounds may dissolve in biological fluids *(2)*. Particles of the same chemical entity (oxides and sulfides) have different biological activity depending on crystalline structure and surface properties *(3,4)*.

Soluble nickel salts include chloride, sulfate and nitrate. Nickel carbonyl ($Ni(CO)_4$) is a volatile, colourless liquid with a boiling-point of 43 °C; it decomposes at temperatures above 50 °C. In biological systems, nickel forms complexes with adenosine triphosphate, amino acids, peptides, proteins and deoxyribonucleic acid.

Sources

Nickel is widely distributed in nature, forming about 0.008% of the earth's crust. The core of the earth contains 8.5% nickel, deep-sea nodules 1.5%; meteorites have been found to contain 5–50% nickel *(5)*.

The natural background levels of nickel in water are relatively low, in open ocean water 0.228–0.693 µg/litre, in fresh water systems generally less than 2

Agricultural soils contain nickel at levels of 3–1000 mg/kg; in 78 forest floor samples from the north-eastern United States of America, concentrations of 8.5–15 mg/kg were reported *(6)*.

The nickel content is enriched in coal and crude oil. Nickel in coals ranges up to 300 mg/kg; most samples contain less than 100 mg/kg but there is a large variation by region *(7)*. The nickel content of crude oils is in the range <1–80 mg/kg *(6,8)*.

Production and use

There are two commercial classes of nickel ore, the sulfide ores (pentlandite and pyrrhotite) and the silicate-oxide. Most nickel is produced from the sulfide ores, and the two largest producers, Canada and the Russian Federation, account for 20–25% each of total annual production, which was 784.82 thousand tonnes in 1988 *(5)*.

Intermediate uses of nickel include 42% in steel production and 36% in the production of other alloys. Electroplating in the form of nickel sulfate accounts for about 18%. The most important end-uses are transportation 23%, chemical industry 15%, electrical equipment 12%, and construction

10% *(6)*. Nickel in coinage, other manufactured products and household appliances may be important for some health effects (dermatitis).
The burning of residual and fuel oils, nickel mining and refining, and municipal waste incineration are the main anthropogenic sources of nickel emissions to the atmosphere *(6)*. These sources account for about 90% of the total global emission, estimated to be 42.85×10^6 kg/year.

The major nickel species in ambient air is nickel sulfate. This soluble ("leachable") form is estimated to comprise 60–100% *(9)*, or 15–93% (average 54%) *(10)* of the nickel components emitted by fly ash from oil-fired utility boilers. The corresponding value for nickel sulfate emission arising from coal combustion is 20–80%. The insoluble fraction of fly ash emitted from both oil and coal combustion exists as nickel oxides and complex metal oxides (ferrites, aluminates, vanadates). Estimates of emission from natural sources vary in the range $8.5–160 \times 10^6$ kg/year *(6)*.

Occurrence in air

Because of the large number of nickel-releasing sources, the nickel concentration in ambient air may show considerable variation. In a remote area (Canadian Arctic) levels of 0.38–0.62 ng/m^3 were recorded *(11)*, as compared to 124 ng/m^3 in the vicinity of a nickel smelter *(12)*. In northern Norway, a level of about 1 ng/m^3 was recorded in an unpolluted area as compared to about 5 ng/m^3 some 5 km distant from a nickel smelter (average values 1990-1991). The highest value recorded was 64 ng/m^3 *(13,14)*. Concentrations of 18–42 ng/m^3 were recorded in 8 United States cities *(15)*. These values correspond to the average value of 37 ng/m^3 for 30 United States Urban Air National Surveillance Network Stations for the period 1957–1968. This average decreased from 47 ng/m^3 for 1957–1960 to 26 ng/m^3 for 1965–1968. The mean (arithmetic) value for 1970–1974 was 13 ng/m^3 *(16)*. Ranges of 10–50 ng/m^3 and 9–60 ng/m^3 have been reported in European cities. Higher values (110–180 ng/m^3) have been reported from heavily industrialized areas *(17)*.

Pentlandite $[(FeNi)_9S_8]$ and nickel in the silicate zone (also called the garnierite zone) are two naturally occurring forms of nickel found in rocks. Nickel from man-made sources is probably represented mostly by oxides and sulfates of rather small particle size (mass median diameter (MMD) about 1 μm) and some 15–90% is soluble (leachable). Occupational studies of nickel exposure have not provided dose-specific estimates of risk for individual species, and only rarely total exposure estimates that are comparable between the different plants.

The MMD of nickel in urban air is 0.83–1.67 μm, and less than 1 μm in 28 55% of particles *(18)*. An MMD of 0.98 μm has also been reported *(19)*. The highest concentration of nickel was found in the smallest particles emitted from a coal-fired plant *(20)*. Particles with an MMD of 0.65–1.1 μm contained nickel at a concentration of 1600 mg/kg, while particles of 4.7–11 μm contained 400 mg/kg. Nickel-containing particles released from oil combustion (California, urban area) are in the fine-size fraction, with MMDs of less than 1 μm *(21)*. Nickel carbonyl has never been demonstrated in ambient air.

Analytical methods

Absorption on cellulose ester membrane filters followed by wet digestion and analysis by electrothermal atomic absorption spectrometry (ET-AAS), inductively coupled plasma atomic emission (ICP-AES) or ICP-mass spectrometry are suitable for analysis of nickel in air, with a detection limit of 5 ng/sample *(22)*.

ET-AAS with Zeeman background correction is currently the most common technique for determining nickel in biological materials. Detection limits of 0.4 mg/litre for urine and 0.05 µg/litre for serum have been reported. It is important to exclude sample contact with nickel-containing materials (e.g. steel syringes) *(23,24)*.

Routes of exposure

The main routes of nickel intake for humans are inhalation, ingestion and absorption through the skin.

Air

Assuming a daily respiratory rate of 20 m^3, the amount of airborne nickel entering the respiratory tract is in the range 0.1–0.8 µg/day when concentrations are 5–40 ng/m^3 in ambient air. Owing to the variation in particle size and solubility between nickel compounds, no general statements can be made on the retention or absorption of nickel in the respiratory tract *(25)*. A total deposition of about 50% of the inhaled dose was estimated for particles with an MMD of 2.0 µm, while deposition was about 10% for those of 0.5 µm. For larger particles, more than 50% of the deposited dose was in the nasopharyngeal part of the respiratory tract as against less than 10% for the smaller particles.

In a single experiment, 95% of the nickel in a respirable aerosol of nickel-enriched fly ash was retained in the lung one month after the exposure *(26)*. Following intratracheal administration of nickel chloride, only 0.1% was retained in the lungs of rats at day 21 *(27)*.

About 0.04–0.58 µg of nickel is released with the mainstream smoke of one cigarette *(6)*. Smoking 40 cigarettes per day may thus lead to inhalation of 2–23 µg of nickel. The possibility that nickel occurs in mainstream smoke in part as nickel carbonyl has never been substantiated.

Drinking-water

Nickel concentrations in drinking-water in European countries of 2–13 µg/litre have been reported *(28)*. An average value of 9 µg/litre and a maximum of 34 µg/litre were recorded in Germany *(29)*. Nickel may, however, be leached from nickel-containing plumbing fittings, and levels of up to 500 µg/litre have been recorded in water left overnight in such fittings *(30)*. In areas with nickel mining, levels of up to 200 µg/litre have been recorded in drinking-water. The average level of nickel in drinking-water in public water supply systems in the United States was 4.8 µg/litre in 1969.

Assuming a concentration of 5–10 µg/litre, a daily consumption of 2 litres of drinking-water would result in a daily nickel intake of 10–20 µg.

Food

In most food products, the nickel content is less than 0.5 mg/kg fresh weight. Cacao products and nuts may, however, contain as much as 10 and 3 mg/kg, respectively *(5)*.

Total diet studies indicate a total average oral intake of 200–300 µg/day *(6)*. Recovery studies indicate an absorption rate of less than 15% from the gastrointestinal tract *(31)*.

Relative significance of different routes of exposure

Percutaneous absorption of nickel is quantitatively minor, but is the most significant for cutaneous manifestations of nickel hypersensitivity *(32)*. Iatrogenic exposure to nickel may occur as a result of dialysis treatment, prostheses and implants, and medication. Such exposure is of minor importance for practical purposes *(33)*. Ear-piercing, however, increases the probability of nickel sensitization *(34)*.

Table 1 summarizes the levels of daily nickel intake by humans from different routes of exposure.

Table 1. Levels of daily nickel intake (µg) by humans from different types/routes of exposure

Type/route of exposure	Daily nickel intake	Absorption
Foodstuffs	<300	45 (<15%)
Drinking-water	<20	3 (<15%)
Ambient air (urban dweller)	<0.8	0.4 (50%)
Ambient air (smoker)	<23	12 (50%)

Both the gastrointestinal and respiratory uptake rates have been estimated on the basis of very limited experimental evidence.

Gastrointestinal uptake is of limited interest for effects other than nickel hypersensitivity. Moreover, even though a low-nickel diet has been reported to improve clinical symptoms in some hypersensitive individuals, other factors seem to be more important.

As the respiratory tract is a major target organ as well as an uptake organ for nickel, inhalation is the most significant route of exposure with regards to lung effects. Retention in the respiratory tract is more important than uptake into the general circulation because respiratory cancer is the critical effect. Given the particle distribution in ambient air, an approximate 50% retention figure seems reasonable for risk estimation. Effects in the lung resulting from oral intake cannot be excluded. Inhibition of 5'-nucleotidase activity and enhanced lipid peroxidation in pulmonary alveolar macrophages have been demonstrated in the respiratory tract following parenteral injection of nickel chloride in rats *(35)*. The relative importance for tumour development of respiratory tract exposure from the general circulation is not known.

Population groups at high probability of exposure

Industrial activity accounts for most of the variability of nickel deposition on the earth's surface, but deposits from meteorites and volcanic eruptions may exceed releases from anthropogenic sources *(36)*. Point-source emission increases nickel exposure, but an impact on health from such emissions has not been convincingly documented *(12,13)*.

Little is known about risk groups in the general population, although smokers and those exposed at work have higher exposures than other groups within the population. Nickel concentrations in workroom air, particularly in the refining industry may be significantly increased compared to those in ambient air. An increased cancer risk has been repeatedly demonstrated in the refining industry,

but not for secondary users of nickel. Workroom air levels of nickel in secondary and end-users of nickel are generally much lower than in the refining industry, often by a factor of 10–100 *(37)*.

Exposure levels in workroom air in the refining industry have been estimated at 1–5 mg/m^3 for soluble nickel, and from less than 2 mg/m^3 to more than 9 mg/m^3 for sulfidic nickel. Exposure to oxidic nickel may have exceeded 10 mg/m^3. In addition, mixed exposures have been the rule rather than the exception. Secondary users of nickel are usually exposed to less than 0.1 mg/m^3 with occasional levels of up to 1 mg/m^3 *(5,33,37)*.

Toxicokinetics

Absorption

At least 50% of a single inhaled dose of nickel carbonyl is absorbed, the agent passing the alveolar wall intact *(6)*.

Few data exist on the absorption of nickel from particulate matter deposited in the respiratory tract. The upper limit for particle retention may be calculated from respiratory deposition and retention models, but such calculations are of limited practical value because of the different biological availability of nickel compounds. Absorption of nickel into the blood may be of limited significance as particles retained in the cells of the respiratory tract are more important.

Soluble nickel compounds are rapidly removed from the lung. For example, Carvalho & Ziemer *(27)* demonstrated that only 0.1% of the dose was found in the lungs 21 days after tracheal instillation of nickel chloride in rats. Menzel *(38)* demonstrated a saturable clearance mechanism of soluble nickel compounds from rat lungs. A steady-state lung burden was observed at a concentration of 90 μg/m^3, as predicted from computer modelling, while the lung burden continued to increase with repeated exposure to 400 μg/m^3. A maximum clearance velocity of 34.6 ng/g of lung tissue per hour was calculated.

Oxidic nickel remains in the lungs following exposure. In golden hamsters exposed to artificial nickel oxide aerosols (unspecified; MMD 1.0–2.5 μm), 20% of inhaled nickel oxide remained after the initial elimination, and 45% of this was still present after 45 days *(39)*. Continuous inhalation (for 6 weeks) of nickel oxide (NiO) at a concentration of 50 μg/m^3 gave rise to comparable figures *(40)*. Wehner et al. *(26)* exposed Syrian hamsters to nickel-enriched fly ash aerosols. Nickel leaching from the nickel-enriched fly ash did not seem to occur to any extent.

Sulfidic nickel takes an intermediate position. In mice, about 10% of an intratracheally administered dose of nickel subsulfide was retained 35 days after the exposure *(41)*.

Oberdörster has considered lung dosimetry at length, using animal–human extrapolation modelling *(25)*. Equivalent human exposure concentrations were calculated on the basis of results in rats *(25,42)*. The model depends heavily on particle size and solubility; further knowledge of the kinetics of inhaled nickel compounds and on mechanisms of clearance and tumorigenicity is needed for reliable modelling and risk estimation.

Humans absorb 15-50% of nickel in drinking-water after an overnight fast compared to less than 15% of that in foods *(31)*.

Distribution

The main carrier protein of nickel in serum is albumin, but nickel is also bound to α-2 macroglobulin and histidine *(24)*.

The body burden of nickel in adult humans averages about 0.5 mg per 70 kg. The highest concentrations of nickel are found in the lung and in the thyroid and adrenal glands (about 20-25 μg/kg wet weight). Most other organs (e.g. kidney, liver, brain) contain about 8-10 μg/kg wet weight *(43)*. Following parenteral administration to experimental animals, the kidney invariably showed the highest concentrations of nickel followed by either the lung or the pituitary glands *(32)*.

Reference values for nickel concentrations in serum and urine from healthy persons without occupational exposure to nickel compounds have recently been compiled *(23,24)*. Values for serum/plasma are in the range 0.14-0.65 μg/litre; values of around 0.2 μg/litre seem to be the most reliable. Corresponding values for urine are 0.9-4.1 μg/litre, with values of 1-2 μg/litre the most reliable. For whole blood, values of 0.34-1.4 μg/litre are given. These values are substantially lower than those reported prior to 1980 because of better analytical methods and improved control of contamination. The metal concentrations in the different samples were not influenced by age or sex. Various diseases (myocardial infarction, acute stroke, thermal burns, hepatic cirrhosis) influence the kinetics of nickel metabolism.

Metabolism and elimination

Nickel may undergo redox metabolism generating the trivalent form thus forming reactive oxygen species. The intracellular release of nickel ion following phagocytosis of particles of oxidic and/or sulfidic nickel is an important metabolic pathway. Minute particles containing nickel have been demonstrated close to the nuclear membrane. Nickel ions may also enter the cell directly, although possible transport mechanisms are unclear.

Parenteral administration of nickel induces changes in the tissue distribution of other metals, and several physiological divalent cations influence nickel metabolism. Specifically, manganese inhibits the dissolution of nickel subsulfide in rat serum, and inhibits phagocytosis of nickel subsulfide particles *(5)*.

Unabsorbed nickel in the gastrointestinal tract is lost in the faeces (reflecting the daily dietary intake). Figures of 180–250 μg/day should be expected on the basis of an estimated daily intake of 200-300 μg and absorption of less than 15%. Excretion of 258 μg/day has been reported *(44)*.

Absorbed nickel is eliminated in the urine. Excretion via sweat, secretion via saliva and deposition in hair have been reported. However, urinary excretion is the main clearance route. The biological half-time of nickel depends on the nickel species tested. For soluble compounds, the half-time of plasma nickel is 11–39 hours in humans; for particulate compounds, half-times of 30–54 hours have been recorded *(33)*. A urinary elimination half-time of 17–48 hours has been reported for the absorbed dose following experimental oral exposure in humans *(31)*. Protracted retention and

gradual elimination from body pools (respiratory organs) take place following exposure to nickel particulates of low solubility *(45)*.

Biomarkers of exposure

Both plasma and urine concentrations of nickel are useful biomarkers of nickel inhalation exposure on a group basis *(33)*. The correlation between exposure and biological values on an individual basis is low and significant only in some investigations involving exposure to soluble compounds.

The levels in plasma and urine are highly dependent on the nickel species in air. High air levels of oxidic and sulfidic nickel give relatively lower plasma and urine values than a corresponding level of soluble chlorides or sulfates, but higher values in the nasal mucosa and probably also in the lungs (possible target organs) *(45,46)*.

Physiologically-based pharmacokinetic modelling

A two-compartment model describing mathematically the whole-body kinetics of the nickel ion has been formulated by Onkelinx & Sunderman *(47)*. Results based on single-injection, continuous-infusion and multiple-dosing experiments using nickel chloride in rats and rabbits revealed a typical two-compartment distribution and an elimination pattern comprising a rapid and a slow clearance phase. The model has been extended to humans with an excellent fit *(31)*. Estimated urinary elimination half-time for absorbed nickel was 28 ± 9 hours.

Menzel *(38,48)* has developed a complete integrated physiologically-based pharmacokinetic model for nickel metabolism based on his inhalation study. In addition to information on lung dosimetry, data pertaining to the kidneys are important because nickel is predominantly excreted in the urine. At a steady state situation with continued intravenous injection, the highest nickel concentration was found in the kidney followed by the lung (ratio about 17:1). The lung value was about two times that of most other organs investigated. For risk assessment, Oberdörster *(25)* has modelled the human lung burden using animal exposure data from Ottolenghi et al. *(42)* as a basis. Inhalation of nickel subsulfide at a concentration of 970 $\mu g/m^3$ in rats was found to be equivalent to inhalation of 4400 $\mu g/m^3$ in humans.

Health effects

There is evidence that nickel is an essential trace element in several animal species, plants and prokaryotic organisms. Nickel appears to be essential for humans, although no data are available concerning nickel deficiency.

Effects on experimental animals and *in vitro* test systems

Toxicological effects

Inhalation of all types of nickel compounds induces respiratory tract irritation, chemical pneumonia, emphysema and varying degrees of hyperplasia of pulmonary cells, and fibrosis (pneumoconiosis) *(5)*. Nickel may precipitate autoimmune phenomena and induce immunosuppression *in vitro*; the clinical importance of such effects has not been reported *(49)*.

Nickel can cross the placental barrier, thus being able to influence prenatal development by direct action on the embryo. Fetal death and malformations have been reported following injection of various species of nickel compounds in experimental animals *(5)*.

Carcinogenic effects

Cancer in experimental animals can be induced by the injection or implantation of nickel compounds in a variety of organs. Nickel subsulfide and ß-nickel monosulfide seem to be the most potent carcinogens in these experiments *(5)*. The production of localized tumours at the site of injection is of limited relevance to occupational or environmental exposure, although some important information on potency has been obtained from such studies. Great differences in carcinogenic potency have been demonstrated, depending on the nickel compound. Some correlations exist between the carcinogenic potency of a given compound and its solubility in biological fluids, its surface oxidation-reduction state, its ability to be phagocytized, and its ability to stimulate erythropoiesis by intravenous injection *(3)*.

Inhalation and ingestion studies are the most relevant for the assessment of potential human risk from environmental exposure to nickel. No ingestion studies are reported and only a few inhalation studies. Ottolenghi et al. *(42)* described a significant increase in the number of lung tumours in rats following inhalation exposure to nickel subsulfide for about 2 years.

Nickel sulfate hexahydrate, green nickel oxide and nickel subsulfide have been tested in 2-year inhalation studies in mice and rats under the United States National Toxicology Program *(50–52)*. No tumorigenic activity was found with any of the compounds in mice or with nickel sulfate in rats. Increases in lung adenomas and carcinomas were found in rats for both the oxide and the subsulfide. The increase was related to the exposure dose but not to the retained dose and subsulfide was the most potent tumorigen, while nickel was retained in the lung to a considerably higher degree after oxide exposure. Both the oxide and the subsulfide also caused an increase in adrenal pheochromocytomas in rats.

Nickel monoxide (not further specified) and metallic nickel dust induced tumours in hamsters following intratracheal instillation. Soluble nickel seems to have a low tumorigenic potential in experimental animals as indicated by the National Toxicology Program study. Inhalation of nickel carbonyl in rats has produced only one lung tumour in three reported experiments compared to none in corresponding control groups *(5)*.

There is no experimental evidence that nickel compounds are carcinogenic when administered orally or cutaneously.

Mutagenic effects and effects identified by other in vitro assays

Negative mutagenicity data were obtained in most bacterial test systems owing to lack of absorption, but many nickel compounds can induce *in vitro* mammalian cell transformation and are clastogenic to various degrees *(5)*.

Critical organs, tissues and effects

The critical organ following inhalation exposure is the respiratory tract. After short-term high-dose inhalation exposure, lung irritation and pneumonia are critical effects. Increased mortality of non-malignant respiratory disease has been reported in nickel refinery workers with more than 5 years of

exposure, and pneumoconiosis has been reported following 12–20 years of exposure. No details on nickel compounds or exposure levels was given, but nickel oxide (Ni_2O_3)was found to be fibrogenic when instilled intratracheally *(6)*. Tumour induction must, however, be regarded as the critical effect.

Ingestion of high doses of nickel salts causes gastric irritation and the skin can be considered as a target organ with dermatitis as a critical effect *(6)*.

The lung is the critical organ following nickel carbonyl inhalation, the effect being pulmonary oedema.

Effects on humans

Toxicological effects

Severe lung damage has been recorded following acute inhalation exposure to nickel carbonyl. Reversible renal effects (in workers), allergic dermatitis (most prevalent in women), and mucosal irritation and asthma (in workers) have been reported following exposure to inorganic nickel compounds *(5)*. Renal effects and dermatitis presumably relate both to nickel uptake by both inhalation and ingestion, in addition to cutaneous contact for dermatitis.

Allergic skin reactions to nickel (dermatitis) have been documented both in nickel workers and in the general population. However, the significance of nickel as a cause of occupationally-induced skin reaction is decreasing. In contrast, there is evidence that nickel is increasingly a major allergen in the general population, especially in women. About 2% of males and 11% of females show a positive skin reaction to patch testing with nickel sulfate. Ear-piercing considerably increases the risk of nickel sensitization *(34)*.

The respiratory tract is also a target organ for allergic manifestations of nickel exposure. Allergic asthma has been reported among workers in the plating industry following exposure to nickel sulfate.

Cytogenic studies have been conducted in workers in the nickel-refining industry (crushing, roasting, smelting and electrolysis), in nickel carbonyl production, and in electroplating. Elevated levels of sister chromatid exchanges and chromosomal aberrations have been demonstrated in workers in nickel refining plants and in nickel platers; the main effect was chromosomal gaps *(5)*. No effects were found in workers exposed to nickel carbonyl *(5)*.

Torjussen et al. *(53)* suggested the use of histopathological changes in the nasal mucosa as a biomarker of effect in refinery workers, but Boysen et al. *(54)* later concluded that such results at best could indicate groups of persons at increased risk for nasal carcinoma.

Carcinogenic effects

Studies linking nickel uptake from the environment and cancer incidence in the general population are not available. There is agreement that nickel refinery workers exposed by inhalation to various nickel compounds in the past are at a significantly higher risk for cancer of the lungs and the nasal cavity than the non-occupationally exposed population *(5,37)*. Laryngeal cancer, kidney cancer, and cancer of the prostate or bone have also been found in nickel workers, but the epidemiological evidence does not indicate a relationship to nickel exposure or to any other occupational origin *(37)*.

At the Clydach refinery, Wales, a high relative risk of nasal and lung cancer has been associated with inhalation exposure in the calcining, roasting and leaching departments before 1920. Much of the risk was related to work at the linear calciner where nickel exposure levels were 10–100 mg/m^3 with a composition of about 60% oxidic, 20% sulfidic, 20% metallic and 3% soluble nickel. Even if the exposure to soluble nickel compounds is low compared to that to the particulate form, analysis indicates that exposure to soluble forms together with the oxidic or sulfidic forms increases the risk. The decrease in nickel air concentrations to a maximum of 20 mg/m^3 in the workplace after 1930 seems to explain the decrease in risk, even if other changes (lower copper and sulfur in the feed) in the production technology also took place *(37)*.

Very high relative risks of nasal and lung cancer have also existed in the calcining, roasting and leaching departments of refineries in Canada (INCO, Ontario) and Norway, (Falconbridge, Kristiansand). Exposure levels in the same range as in Clydach were recorded in Canada, somewhat lower (>10 mg/m^3) in Norway *(37)*. In Norway, oxidic nickel was reduced to concentrations of less than 5 mg/m^3 after 1955, but an increased risk of lung cancer was still recorded in a cohort with first job entry after 1956 *(55)*. In a recent follow-up, the highest risk was found among those with the highest estimated dose of soluble nickel. There appeared to be a multiplicative effect for smoking and exposure to total nickel *(56)*.

Studies of industrial secondary and end-users of nickel have generally not shown carcinogenic effects, but the exposure levels have been less than 1 mg/m^3 both for particulate and for soluble nickel compounds.

Evaluation of human health risks

Exposure evaluation

Nickel is present throughout nature and is released into air and water both from natural sources and as a result of human activity.

In nonsmokers, about 99% of the estimated daily nickel absorption stems from food and water; for smokers the figure is about 75%. Nickel levels in the ambient air are in the range 1–10 ng/m^3 in urban areas, although much higher levels (110–180 ng/m^3) have been recorded in heavily industrialized areas and larger cities. There is, however, limited information on the species of nickel in ambient air.

Consumer products made from nickel alloys and nickel-plated items lead to cutaneous contact exposure.

Exposure to nickel levels of 10–100 mg/m^3 have been recorded for occupational groups, with documented increased cancer risk. Exposure levels in the refining industry are currently usually less than 1–2 mg/m^3, often less than 0.5 mg/m^3. Experimental and epidemiological data indicate that the nickel species in question is important for risk estimation.

Health risk evaluation

Allergic skin reactions are the most common health effect of nickel, affecting about 2% of the male and 11% of the female population. Nickel content in consumer products and possibly in food and

water are critical for the dermatological effect. The respiratory tract is also a target organ for allergic manifestations of occupational nickel exposure.

Work-related exposure in the nickel-refining industry has been documented to cause an increased risk of lung and nasal cancers. Inhalation of a mixture of oxidic, sulfidic and soluble nickel compounds at concentrations higher than 0.5 mg/m^3, often considerably higher, for many years has been reported *(37)*.

Nickel has a strong and prevalent allergenic potency. There is no evidence that airborne nickel causes allergic reactions in the general population, although this reaction is well documented in the working environment. The key criterion for assessing the risk of nickel exposure is its carcinogenic potential.

In general, nickel compounds give negative results in short-term bacterial mutagenicity tests because of limited uptake. However, they show a wide range of transformation potencies in mammalian cell assays, depending mainly on their bioavailability.

Both green nickel oxide and the subsulfide have caused tumours in animal inhalation studies. In addition, nickel monoxide (not further specified) and an alloy with 66.5% nickel and 12.5% chromium caused tumours following tracheal instillation. A corresponding instillation with an alloy of 26.8% nickel and 16.2% chromium had no such effect, indicating that it was nickel and not chromium which caused the tumours. Injection-site tumours in a number of organs are found with many particulate nickel compounds. The tumorigenic potency varies with chemical composition, solubility and particle surface properties *(57,58)*.

Epidemiological evidence from the nickel-refining industry indicates that sulfidic, oxidic and soluble nickel compounds are all carcinogenic. Exposure to metallic nickel has not been demonstrated to cause cancer in workers.

Several theories have been suggested for the mechanisms of nickel tumorigenesis. All of these assume that the nickel ion is the ultimate active agent. On the basis of the underlying concept that all nickel compounds can generate nickel ions which are transported to critical sites in target cells, IARC classified nickel compounds as carcinogenic to humans (group 1) and metallic nickel as possibly carcinogenic to humans (group 2B) *(5)*.

On the basis of one inhalation study *(42)*, the US Environmental Protection Agency (EPA) classified nickel subsulfide as a class A carcinogen and estimated the maximum likelihood incremental unit risk to be $1.8–4.1 \times 10^{-3}$ *(59)*. However, this study involves only exposure to nickel subsulfide. It is not known whether this compound is present in ambient air, but since it is probably one of the most nickel potent compounds, this risk estimate may represent an upper limit, if accepted. WHO estimated an incremental unit risk of 4×10^{-4} $(\mu g/m^3)^{-1}$ calculated from epidemiological results *(60)*.

On the basis of epidemiological studies, EPA classified nickel dust as a class A carcinogen and estimated the lifetime cancer risk from exposure to nickel dust to be 2.4×10^{-4}. This estimate placed nickel in the third quartile of the 55 substances evaluated by the EPA Carcinogen Assessment

Group with regard to their relative carcinogenic potency *(61)*. Assuming a content of 50% of nickel subsulfide in total dust, a unit risk of 4.8×10^{-4} was estimated for this compound.

An estimate of unit risk can be given on the basis of the report of lung cancer in workers with first employment in 1968–1972 followed through to 1987 in Norway *(55,56)*. Using the estimated risk of 1.9 for this group and an exposure of 2.5 mg/m^3, a lifetime exposure of 155 μg/m^3 and a unit risk of 3.8×10^{-4} (μg/m^3)$^{-1}$ can be calculated.

Guidelines

Even if the dermatological effects of nickel are the most common, such effects are not considered to be critically linked to ambient air levels.

Nickel compounds are human carcinogens by inhalation exposure. The present data are derived from studies in occupationally exposed human populations. Assuming a linear dose– response, no safe level for nickel compounds can be recommended.

On the basis of the most recent information of exposure and risk estimated in industrial populations, an incremental risk of 3.8×10^{-4} can be given for a concentration of nickel in air of 1 μg/m^3. The concentrations corresponding to an excess lifetime risk of 1:10 000, 1:100 000 and 1: 1 000 000 are about 250, 25 and 2.5 ng/m^3, respectively.

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Review

Molecular determinants of responses to myocardial ischemia/reperfusion injury: focus on hypoxia-inducible and heat shock factors

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Abstract

During the past several years much new evidence has accumulated regarding the molecular and biochemical mechanisms underlying cardiac responses to hypoxia and to ischemia/reperfusion injury. Studies have involved cell culture, and ex vivo and in vivo preparations. This review focuses on regulation of two transcription factors that are thought to be important in these processes, hypoxia-inducible factor1α (HIF-1α) and heat shock factor (HSF). Both of these molecules are expressed acutely and chronically in response to hypoxia and ischemia/reperfusion, and both have numerous targets that comprise part of integrated response to ischemic injury aimed at promoting cell survival. Emphasis is placed on new mechanisms of action that regulate HIF-1α, HSF, and heat shock proteins as key responses to hypoxia and ischemia, and possible approaches to therapy based on these data are discussed.

Keywords: Hypoxia; Reoxygenation; Ischemia/reperfusion; Gene regulation; Transcription factor; Preconditioning; Hypoxia-inducible factor (HIF)

1. Introduction

Ischemic injury to the myocardium due to the spectrum of coronary occlusion events known as acute coronary syndromes account for a large proportion of all hospital admissions and of all causes of death in western society. Despite several recent successful advances in the therapy of acute coronary syndromes based on limiting the extent of myocardial damage that ensues following occlusion of a major coronary artery by rapid restoration of blood flow, many patients are not suitable candidates for such revascularization procedures. Additionally, these approaches often are applied too late to prevent irreversible damage to the myocardium. Thus, a greater understanding of the cellular and molecular mechanisms involved in ischemic injury may foster additional improvements in clinical care.

Oxygen is one of the basic elements that supports all eukaryotic life, acting as the final electron acceptor in the respiratory chain. Intracellular O_2 concentrations are maintained within a narrow range due to the risk of oxidative damage from excess O_2 (hyperoxia), and of metabolic demise from insufficient O_2 (hypoxia). Because insufficient levels of oxygen deprive the respiratory chain of its main electron acceptor, the less efficient anaerobic glycolytic pathway supersedes mitochondrial oxidative phosphorylation as the principal source of ATP production, generating approximately a quarter of the amount of ATP normally produced during oxidative phosphorylation [1]. As a result, there are inadequate levels of high-energy phosphates to maintain normal function. If hypoxia is reversed, the subsequent reoxygenation may potentially produce damage to cells by increasing the levels of reactive oxygen species (ROS) generated during partial reduction of oxygen to water.

The heart is an organ with particular susceptibility to hypoxia since only limited reserves of high-energy phosphates are maintained [2]. The myocardium may be exposed to hypoxia or anoxia under a number of conditions such as myocardial ischemia after major coronary artery occlusion,

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high altitude, and anemia. The extent as well as duration of hypoxia, in addition to the presence of other confounding factors such as tissue ischemia, determines the cardiac response to diminished oxygen supply. Both hypoxia and oxidative stress result in biochemical and functional changes as the heart attempts to maintain function in the face of perturbations in oxygen tension. Hypoxia and reoxygenation alter the cardiac protein pattern, mainly through altered gene expression, but also through changes in mRNA stability, rates of translation, post-translational protein modifications and degradation. Hypoxia also induces upregulation of specific proteins that lead to both protective as well as deleterious effects. Furthermore, increased oxygen levels occur upon reperfusion of an occluded coronary vessel (reoxygenation), either spontaneously or following treatment of acute myocardial infarction. Such reperfusion may be associated with pathophysiological levels of reactive oxygen species. Subsequently, cardiac myocytes attempt to protect themselves from the short- and long-term consequences of exposure to these harmful molecules by upregulating levels of antioxidant and stress proteins. Numerous experiments indicate that levels of superoxide increase within the hypoxic myocardium during production of H_2O_2 and hydroxyl radicals upon reoxygenation [3]. Under many clinical circumstances, there is a chronic reduction in coronary blood flow as occurs in the border zone surrounding a myocardial infarction. Such alterations in coronary perfusion have also been documented in patients with native vessel chronic coronary artery obstructions, bypass graft stenosis, and restenosis after coronary angioplasty/stent procedures. These alterations in coronary blood flow may lead to either global or regional myocardial ischemia, which can be repetitive, and can cause long-term homeostatic responses as outlined in Table 1.

Although the maintenance of oxygen homeostasis is an essential cellular and systemic function, it is only within the past several years that the molecular mechanisms underlying this fundamental aspect of cell biology have begun to be elucidated and their connections to physiology and pathophysiology have been established. Thus, the purpose of this review is to update the reader on the rapidly advancing knowledge concerning the regulatory mechanisms involved in the regulation of hypoxia-inducible transcription factor(s) and heat shock factor in ischemic myocardium.

Table 1
Direct HIF-1 target genes

Erythopoeisis and iron metabolism
Ceruloplasmin, erythropoietin (EPO), transferrin, transferrin receptor
Vasculogenesis/vasomotor tone
α1B Adrenergic receptor, adrenomedullin, endothelin-1, heme oxygenase-1
Nitric oxide synthase (NOS) 2, plasminogen activator inhibitor 1, vascular endothelial growth factor (VEGF), VEFG receptor Flt-1
Prosurvival/proliferation factors
Adrenomedullin, cyclin G2, EPO, heme oxygenase-1. IGF2, IGFBP-1,2,3 NOS2, NIP3, p21, TGF-β3, VEGF
Metabolism
Adenylate kinase-3, aldolase A/C, enolase 1, carbonic anhydrase-9, glucose transporter (GLUT)-1/3, 11 glycolytic enzymes

1.1. Hypoxia-sensing mechanisms

In mammalian cells, many of the compensatory mechanisms that occur in response to changes in oxygen tension are secondary to inhibition of oxygen-dependent pathways and changes in the intracellular redox status. However, some cellular responses to hypoxia become activated before either ATP levels are depleted or the K_m of the mitochondria for O_2 is reached [4–6]. Moreover, the mitochondrial inhibitor cyanide does not reproduce some hypoxia-induced effects. Based on previous biochemical observations, the oxygen-sensing molecule appears to be a heme-containing protein that binds to oxygen; is inhibited by carbon monoxide; and is activated by certain heavy metals, notably cobalt, nickel, and manganese, which can substitute for iron in heme proteins [7,8]. Until recently, the means by which cells sense alterations in oxygen tension remained relatively obscure. The first insight into an oxygen-sensing pathway in higher organisms came with the discovery of a family of oxygen-dependent enzymes responsible for the regulation of the hypoxia-inducible transcription factors (HIFs). Activation of the HIF system by iron chelators and cobaltous ions as well as hypoxia has led to the proposal of a ferroprotein oxygen sensor regulating mechanism for HIF transcription factors.

The HIF transcription factors are composed of two subunits: the hypoxia-regulated alpha subunit HIF-1α (or its homologs, HIF-2α and HIF-3α), and the oxygen-insensitive HIF-1β subunit (also known as the arylhydrocarbon receptor nuclear translocator, or ARNT) [9]. Under normal oxygen conditions (normoxia), HIF-1α is constitutively expressed [10–12]. However, this subunit is rapidly targeted for proteasome-mediated degradation through a protein–ubiquitin ligase complex containing the product of the von Hippel Lindau tumor suppressor protein (pVHL) [13–16]. Under hypoxic conditions, degradation of HIF-1α is prevented, and thus HIF-1α is able to accumulate within the nucleus allowing it to bind with its partner HIF-1β (see Fig. 1). Subsequently, this heterodimeric complex is able to recognize HIF-responsive elements (HREs) transactivating downstream target genes involved in the longer-term response to chronic hypoxia [17] (see Fig. 1 and Table 1).

Recently, it has been reported that degradation of HIF-1α under nomoxic conditions is triggered by post-translational hydroxylation of conserved proline residues within a polypeptide region known as the oxygen-dependent degradation domain (ODD) [18,19]. The hydroxylated proline residues in this sequence are recognized by pVHL, leading to subsequent HIF-1α degradation via the ubiquitin ligase pathway (Fig. 1). This modification is inherently oxygen-dependent, because the oxygen atom of the hydroxyl group is derived from molecular oxygen. Moreover, this reaction

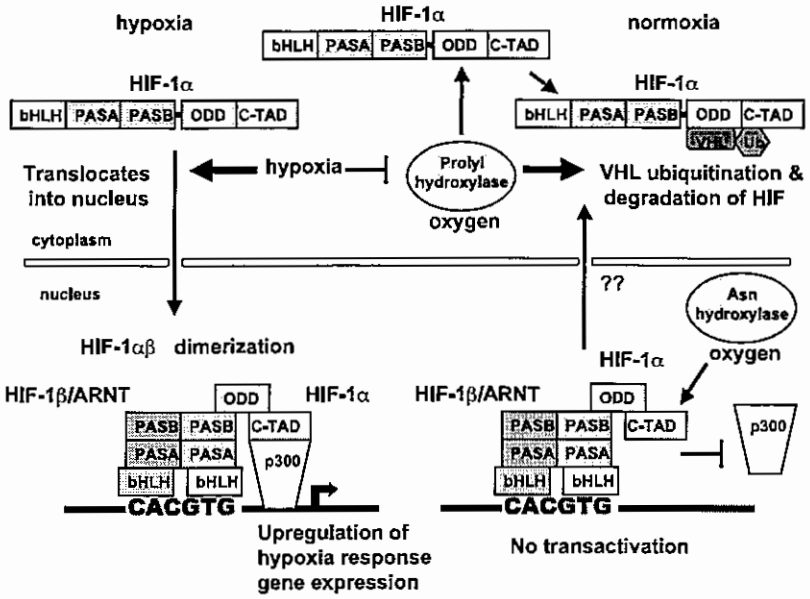

Fig. 1. Regulation of HIF-1 activity by cellular oxygen concentration. In the cytoplasm, the oxygen-dependent degradation (ODD) domain of HIF-1α is modified by a HIF-prolyl hydroxylase under normoxic conditions. This post-translational modification leads to HIF-1α recognition by pVHL (product of the von Hippel Lindau tumor suppressor protein), and subsequent degradation by proteasome ubiquination (Ub) processes. While in the nucleus during normoxia, an asparaginyl (Asn) hydroxylase modifies the C-TAD domain of HIF-1α, subsequently blocking its binding with the transcriptional coactivator p300. Hypoxia blocks both prolyl hydroxylation and asparaginyl hydroxylation, allowing HIF-1α to accumulate in the nucleus as well as to bind to p300 This interaction with p300 allows for transcription of downstream HIF-1 target genes and thereby enabling cells and the whole organism to adapt to hypoxia. For more details, see text.

requires cofactors such as 2-oxoglutarate, vitamin C, and iron. The requirement of this last cofactor is consistent with previous biochemical evidence suggesting the oxygen-sensing factor is iron-dependent. Thus, this critical regulatory event is carried out by a family of iron (II)-dependent dioxygenase prolyl hydroxylase enzymes that use O_2 as a substrate to catalyze hydroxylation of the target proline residues [20]. Because oxygen appears to be rate limiting for prolyl hydroxylase activity, these enzymes likely represent the oxygen sensors that provide a direct link between oxygen concentrations and components of the hypoxic response pathway.

Modulation of protein stability is one pathway by which HIF activity is regulated by hypoxia. However, more recent studies have identified an additional novel mechanism by which HIF is regulated by an oxygen-dependent enzyme [21]. In addition to the ODD domain, the HIFα subunit isoforms contain two transactivation domains responsible for recruiting transcriptional coactivators essential for gene expression: (1) the N-terminal transactivation domain (N-TAD), which overlaps the ODD. Regulation of its activity is likely to be a byproduct of protein stability; and (2) the C-terminal transactivation domain (C-TAD), which is able to recruit coactivator complexes such as p300/CBP only under hypoxic conditions [22–24] (Fig. 1). Earlier studies revealed that transcriptional activation by C-TAD, when fused to a heterologous DNA-binding domain, is able to operate independently of the ODD, recruiting coactivator complexes such as p300/CBP only under hypoxic conditions. Surprisingly, the regulation of C-TAD activity also involves an oxygen-dependent hydroxylation event; however, in this case, the targeted residue appears not to be a proline but rather a conserved asparagine residue. Enzymatic hydroxylation of the conserved C-TAD asparagine residue (Asn-803) requires oxygen; this modification subsequently prevents the recruitment of coactivators such as p300 and CBP. The enzyme responsible for this modification is factor-inhibiting HIF-1 (FIH1), which has been previously demonstrated to bind to HIF-1 and to repress HIF-dependent transcription [25]. FIH1, like members of the proline hydroxlyases, is a member of the 2-oxoglutarate and iron-dependent dioxygenase superfamily [26].

Thus, study of the interaction of pVHL with HIF has led to new insights into the mammalian oxygen-sensing pathway and has demonstrated an unappreciated role for enzymatic protein hydroxylation in intracellular signaling. The discovery of this modification, as well as identification of the responsible enzymes, raises the question of whether this modification occurs on other intracellular proteins. Additionally, it will be interesting to determine if additional members of the 2-oxogluturate and iron-dependent dioxygenase superfamily and thus potential protein hydroxylases exist [21]. The answers to such questions may provide additional new insights into novel hypoxia-mediated pathways.

Additionally, recent studies have provided experimental evidence in support of the hypothesis that mitochondrial generation of superoxide and, subsequently, hydrogen per-

oxide are required for induction of HIF-1 activity and transcription of downstream target genes in hypoxic cells [27]. This model proposes that reactive oxygen species generation increases under hypoxic conditions. However, an alternative model proposes the opposite, that hypoxia results in decreased production of ROS by nicotinamide adenine dinucleotide phosphate (reduced NADPH) oxidases. It is likely that HIF-1α is a direct target of redox regulation. The redox status of cysteine residues of HIF-1α in the carboxy-terminal TAD (TAD-C) has been shown to affect its interaction with CBP/p300 coactivators, and this interaction is positively regulated by redox factor 1 (REF-1) and thioredoxin [22]. In addition, the interaction of the coactivator SRC-1 with HIF-1α is also redox regulated [28]. However, these models are supported by experimental data derived primarily from the use of redox-sensitive fluorescent compounds that measure reactive oxygen species (ROS) and from the effects of pharmacologic agents on the expression of HIF-1 and downstream genes in tissue culture. Given the lack of consensus on the role of ROS on HIF-1 activity, further in vivo genetic approaches will be required to determine the function of ROS on hypoxia-mediated signal transduction [27]. Additionally, the regulation of hypoxia by carbon monoxide (CO) and nitric oxide (NO) also is likely mediated through HIF-1 activity [29]. In one such recent report, it was found that cardiac protection induced by intermittent hypoxia is critically dependent on *Hif1α* gene dosage [30].

Despite the recent exciting findings of oxygen-sensing proteins regulating HIF-1 activity, the regulation of HIF-1 is a complex process likely involving not only O_2 concentration and oxygen-sensing proteins, but also HIF-1 protein stabilization via the PI3K/PTEN/Akt/FRAP/p70S6 kinase prosurvival pathway [31]. Multiple signaling pathways have been found to stabilize the HIF-1 transcriptional complex through the Akt pathway including nitric oxide, HER2/Neu, IGF/insulin, and erythropoietin [32–35]. Stretch-activated channels in the myocardium have also been implicated in this pathway [36]. However, the PI3 3-kinase/Akt pathway, which may also involve mTOR, is neither sufficient nor necessary to induce HIF-1 activity by itself, but may modulate HIF activity under hypoxic conditions [37]. Moreover, the cardioprotective effects of the Akt pathway may also act independently of HIF-1 activity [38]. Interestingly, many of the signaling factors which activate the Akt pathway are direct targets of the HIF-1 transcriptional regulation, such as erythropoietin and IGF2 (see Table 1 and Fig. 2). Alternatively, the Akt pathway may not directly regulate HIF-1 stability, but rather HIF-1 may act upstream of Akt to promote cardiac survival through other pathways.

Although HIF-1 mRNA and protein are detected in cell culture models hours after the induction of hypoxia [39] and days after myocardial infarction [40], much earlier responses have also been documented in animal models [41]. For example, Chun et al. recently reported that HIF-1α protein accumulated in nuclei of adult rat cardiac myocytes as early as 2 h after regional ischemia [42]. The raised level of HIF-1α was related to the activation of the promoter for atrial natriuretic factor (ANF) gene, suggesting that acute increases in ANF in the left ventricle after ischemic injury is HIF-1α-dependent [42]. These observations are consistent with the virtually instantaneous hypoxic induction of HIF-1α and its reduced degradation in the presence of hypoxia [43].

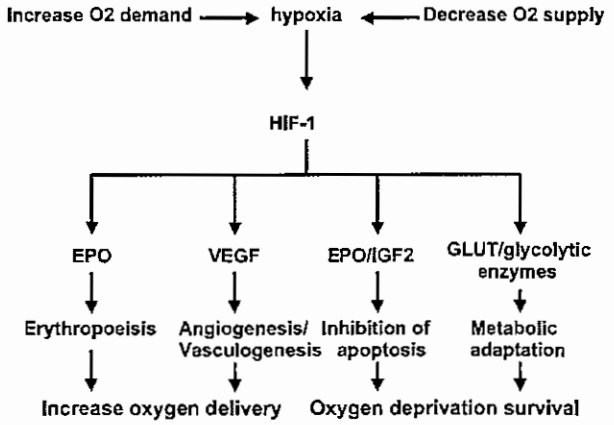

Fig. 2. HIF-1 downstream activators and hypoxia adaptive responses. Activation of the HIF-1 pathway leads to transcription of target genes, which activate pathways that increase oxygen delivery and promote adaptive prosurvival responses. EPO—erythropoeitin, VEGF—vascular endothelial growth factor, IGF2—insulin growth factor 2, GLUT—glucose transporter.

Although glucose as well as GLUT1 overexpression diminished HIF-1α in rat neonatal cardiac myocytes subjected to 24 h of hypoxia [39], 2 h of reperfusion following 20 min of ischemia in isolated rat hearts perfused with a high glucose solution resulted in a marked increase in HIF-1α mRNA [44]. These reports and others cited above [40–42] indicate that HIF-1α responses are present over a broad spectrum of time following ischemia/reperfusion injury, but also suggest that acute responses could be the result of free radical generation. Future experiments will be necessary to explore this hypothesis. Thus, continued cellular and molecular research will likely reveal additional novel pathways regulating the HIF-1α response to hypoxia and ischemia.

1.2. Heat shock factors in the ischemic myocardium

The heat shock response is a highly conserved defense mechanism against tissue and cell stress injury. This system is conserved from bacteria to humans, and represents an endogenous mechanism that antagonizes protein unfolding or misfolding during stress responses. This defense mechanism requires heat shock transcription factors (HSFs), the primary mediators of the heat shock response. When cells are exposed to stress, HSFs are phosphorylated and form trimers that enter the nucleus and bind the heat shock elements (nGAAn) within the promoter/enhancer regions of heat shock proteins (HSPs) [45].

There are at least four known heat shock transcription factors present in mammalian cells; however, HSF1 appears to be the primary mediator of the heat shock response system. The other three heat shock transcription factor isoforms (HSF2-4) are not sufficient to preserve the heat shock response in the absence of HSF1 [46]. Disruption of the *Hsf1* gene in mice reduces cardiac expression of Hsp25, αβ-crystallin and Hsp70, but not Hsp60 and Hsp90. Consistent with the downregulation of Hsp25 was decreased activity, but not protein content, of glucose 6-phosphate dehydrogenase. Consequently, superoxide was generated at a higher rate, and several mitochondrial proteins underwent greater oxidation as a result of in vivo HSF1 deficiency. Overall, these results indicate loss of the *Hsf1* gene in mice eliminates the heat shock response, and increases the susceptibility of HSF1-deficient cells to oxidative damage at normal (37 °C) temperature [47].

HSF1 activation during ischemia may be induced by multiple cellular stress responses. A decrease in the concentration of high-energy phosphate compounds may be sufficient to activate HSF1 [48]. Intracellular acidosis may also serve as an additional stimulus. Alterations in redox state have been documented to activate cardiac HSF1 DNA binding [49], and activate HSF1 acutely during ischemia/reperfusion [50]. However, it appears that a common consequence of stimuli that activate HSF1 is an increase in the concentrations of unfolded proteins within the cell, which may provide a common stimulus for induction of HSP gene expression [51]. Negative regulation of HSF1 may be due to several mechanisms during unstressed conditions. Interestingly, HSF4 has been suggested to negatively regulate the expression of the *Hsf1* gene, and possibly, the overall heat shock response [52]. Another negative regulator of HSF1-mediated transcription is glycogen synthase kinase 3β, which also impairs HSF1 DNA binding [53].

Reperfusion of the ischemic rat heart causes rapid activation of HSF1 [54]. A characteristic feature of the response to stress in human (but not rodent) cells is rapid and reversible relocalization of HSF1 within seconds into specific subnuclear stuructures, termed stress granules [55], where trimerization occurs. This process also involves HSF2 and coincides with the nucleolar localization of Hsp70 [56]. The appearance of stress granules correlates with the inducible phosphorylation and transcription activation of HSF1 [55]. Upon recovery from stress such as heat shock, HSF1 rapidly dissipates from these stress granules to a diffuse nucleoplasmic distribution, typical of unstressed cells [55]. Although the majority of HSF1 phosphorylation sites are still unknown [57], it has been shown that phosphorylation of serine 230 promotes inducible transcriptional activity of HSF1 [58]. In stress granules, HSF1 undergoes post-translational modification by covalent conjugation of a small ubiquitin-like modfifier 1 protein (SUMO-1) to lysine 298 [59] preceded by phosphorylation of serine 302 [57]. Negative regulators of HSF1 driven transcription include the mitogen activated protein kinase ERK and c-Jun NH2-terminal kinase [60]. These kinases recognize their substrates via a small domain (D domain) in which phosphorylation of serine 363 appears to be the major target leading to reduced transcriptional activity [60].

1.3. Heat shock proteins in cardioprotection

Heat shock proteins (HSPs) fulfill a range of functions, including cytoprotection and the intracellular assembly, folding, and translocation of oligomeric proteins [61], and represent a rapid response to altered redox states [49]. HSPs are categorized into several families identified on the basis of their approximate molecular weights, which range from 10 to 150 kDa. Members of the HSP family are induced in response to a number of stresses, including sublethal heat, hypoxia, reoxygenation after hypoxia, and ischemia. They function to promote the folding and assembly of nascent polypeptides, and to facilitate the repair or degradation of unfolded proteins [51,62,63]. In addition to acting as cellular chaperones, HSPs mediate cytoprotection by associating with and hindering the action of key apoptotic proteins, and by facilitating the degradation of misfolded intracellular proteins by the ubiquitin/proteasome system, so-called "protein triage" [64].

Thus, numerous studies over the past decade have demonstrated that increased expression of HSPs may protect the heart from stressful environments such as ischemia and reperfusion injury [65,66]. Hearts isolated from transgenic mice that express human HSPs such as HSP70 in the myocardium have shown greatly improved functional recovery, with decreased infarct size after experimental induction of ischemia and reperfusion [67–69]. The role of HSP70 in myocardial protection has been extensively studied [70,71].

HSP70 is present in two forms: constitutive (HSP70c) and inducible (HSP70i). Both forms can be activated by stress and there is evidence in H9c2 cardiac myoblasts that the constitutive form confers protection against oxidative injury [72]. Stable overexpression of HSP70c in these cells also confers oxidative protection [73]. In other experiments, it was reported that both the inducible and the constitutive forms of this protein were activated by ethanol or heat resulting in a decrease in cytotoxicity produced by oxidative stress [74]. Previous studies had shown that overexpression of rat HSP70i increased cardiac resistance to ischemic injury [69]. In this same transgenic model, a short period of ischemia followed by reperfusion sufficient to cause regional dysfunction without infarction protected against left ventricular dysfunction compared to wild-type littermate controls [75]. Direct gene delivery by cardiac injection with recombinant adenovirus encoding HSP70i reduced infarct size in vivo after ischemia/reperfusion in the rabbit heart [76]. Delayed cardioprotection can be induced via κ-opioid receptor activation in adult rat ventricular myocytes. Zhou et al. [77] showed that lethal-simulated ischemia activated both HSP70c and HSP70i, but that only the antisense

oligonucleotide to HSP70i blocked delayed cardioprotection. Thus it is reasonable to hypothesize that under some experimental circumstances HSP70i is the predominant isoform of this molecular chaperone involved in conferring cardioprotection.

HSPs other than HSP70 may also provide added myocardial protection (Fig. 3). These factors include the larger heat shock proteins HSP60 and HSP90 and the small heat shock proteins HSP22, HSP27, αβ-crystallin, and HSP32. Increased expression of HSP27 in canine cardiac myocytes correlated with a greatly decreased cardiomyocyte susceptibility to metabolic or functional injury after simulated reperfusion injury [78]. Overexpression of HSP27 and αβ-crystallin protected adult rat cardiac myocytes against ischemic injury [79] and transgenic overexpression of αβ-crystallin conferred simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion [80].

Mechanisms of cytoprotection conferred by activation of αβ-crystallin include preservation of the tubulin cytoskeletal structure against ischemia-induced disruption [81], redistribution of αβ-crystallin from the cytosol to intercalated disks and Z lines of the myofibrils to provide stabilization of the myocardial contractile apparatus [82], and binding to the I-band portion of titin, an important elastic component of the myofibrils [83]. αβ-Crystallin negatively regulates cytochrome *c*- and caspase-8-dependent activation of caspase-3 [84] and further controls apoptosis by regulating Akt activation [85]. Like HSP70, phosphorylation plays a major role in HSP27 and αβ-crystallin regulation. Thus Akt phosphorylates HSP27 on serine-82 resulting in its dissociation from Akt. Activation of MKK6 stimulates p38 MAPK and results in the induction of αβ-crystallin mRNA and phosphorylation of αβ-crystallin on serine-59 [86]. This phosphorylation is both necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis [87]. It has also been shown that mutation of COOH-terminal lysines in overexpressed αβ-crystallin abrogates ischemic protection in cardiomyocytes [88].

HSP22 is a newly described member of the small heat shock protein superfamily and interacts with a mimic of phosphorylated HSP27; its physiologic role remains to be determined [89]. HSP32 (heme-oxygenase-1 or HO-1), which has received relatively little attention, is increased by hypoxia in rat neonatal myocytes [90] and is an effective antioxidant cardioprotective molecule [91]. HO-1 is negatively regulated by the transcriptional repressor Bach1 [92]. A physiologically regulated vector expressing the human HO-1 gene conferred protection against cardiac ischemia–reperfusion injury in a rat model [93].

HSP60 and HSP10 are heat shock proteins found predominantly in the mitochondria. Accumulation of unfolded proteins within the mitochondrial matrix results in the transcriptional upregulation of nuclear genes encoding these stress proteins via a specific transcription factor termed CHOP [*C*/EPB *ho*mology *p*rotein] [94]. In rat neonatal myocytes, overexpression of HSP60 and HSP10 separately or together protected cells against apoptosis induced by simulated ischemia and reoxygenation [95]. This protection was accompanied by increased ATP recovery and preservation of complex III and IV activities in mitochondria [95]. Only 15–20% of HSP60 is found in the cytosol. However, HSP60 complexes with cytosolic bax, and reduction of HSP60 by an antisense approach precipitated translocation of bax to the mitochondria and apoptosis in adult rat cardiac myocytes resulting in the release of cytochrome *c*, activation of caspase 3, and induction of DNA fragmentation [96]. In this cell preparation, hypoxia had a similar effect resulting in the dissociation of the HSP60–bax complex in the cytosol with translocation of cytosolic HSP60 to the plasma membrane and bax to the mitochondria, a process sufficient to trigger apoptosis [97]. These changes occur

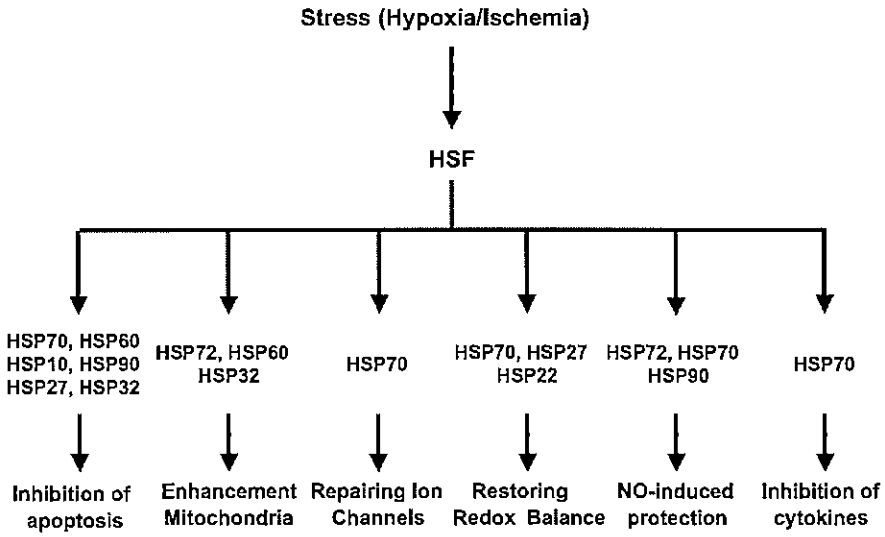

Fig. 3. Heat shock response pathways and hypoxia adaptive responses.

before reoxygenation and the concomitant generation of free radicals [97]. How ischemic preconditioning and other cardioprotective maneuvers regulate HSP60 and HSP10 has not been determined.

HSP90 is an ATP-dependent molecular chaperone involved in the folding and activation of a large number of substrate proteins that include protein kinases and transcription factors such as HIF-1α and HSF1 [98,99]. As substrate proteins interact with HSP90, multiprotein complexes are formed with a set of highly conserved partner proteins, such as HSP70, HSP organizing protein (HOP), HSP40, and p23 [98,99]. ATP hydrolysis by an intrinsic ATPase results in a conformational change in HSP90 that is required to induce conformation change in the substrate or "client" protein [99]. The mechanisms of these interactions have recently been reviewed in detail [99]. It has been observed that p23 and HSP90 can also be involved in the disassembly of transcriptional regulatory complexes [100].

Trimeric human HSF1 associates with an HSP90–immunophilin–p23 complex through its regulatory domain resulting in transcriptional repression of trimeric HSF1 [101]. Following stress, this heterocomplex dissociates, triggering HSF1 activation and heat shock gene transcription. This response depends in part on the small GTPase Ral [102]. A prototype receptor regulated by HSP90 is the steroid receptor, in which HSP90 induces a conformational change allowing binding of the steroid receptor [99]. In adult rat cardiac myocytes, steroid hormones via the interaction of HSF1 and HSP90 regulate heat shock protein expression [103]. An inhibitor of HSP90, radicicol, reduced hypoxia-induced VEGF expression by inhibiting the interaction between HIF-1α/aryl hydrocarbon receptor nuclear translocator (Amt) heterodimer and the hypoxia response element [104]. Further evidence that HSP90 is involved in the regulation of HIF-1α was reported by Isaacs et al. [105] who showed that disruption of HSP90 function by the HSP90 inhibitor geldanamycin promoted HIF-1α degradation via a novel, oxygen-independent E3 ubiquitin ligase and diminished HIF-1α transcriptional activity. This resulted in a marked reduction in both accumulation of hypoxia-induced VEGF mRNA and hypoxia-dependent angiogenic activity [105]. Both cardiotrophin-1, a member of the interleukin-6 family of cytokines, and urocortin, a 40-amino-acid peptide that belongs to the corticotrophin-releasing factor family of peptides which mediate the action of the hypothalamic–pituitary–adrenal axis in response to stress, increase the expression of HSP90 and are cardioprotective in cardiac myocytes [106,107]. Thus, HSP90 is a multifunctional HSP that is involved in a large number of stress responses in the cardiovascular system by its interaction both with other molecular chaperones and by its regulation of both HIF-1α and HSF.

The reports cited above indicate the complexity of the mechanisms responsible for the myocardial protection provided by HSPs which remain incompletely understood. As indicated above, recent studies have proposed multiple roles for HSPs in myocardial protection ranging from direct cytoprotection of myocardial cells against reactive oxygen species (ROS) during ischemia and reperfusion to modulation of cytokine activity (Fig. 3). Thus, HSPs may favorably interfere with ROS-induced phenomena during ischemia and reperfusion because of their biologic roles as molecular chaperones. HSP levels closely correspond to the activity of antioxidant enzymes such as catalase [63]. Furthermore, cytokine production may be downregulated by HSPs through interference with the nuclear factor κB (NFκB) signalling pathway which is involved in inducing several proinflammatory genes/cytokines [108–110].

Finally, accumulating evidence from in vivo and in vitro studies strongly suggests that the heat shock response system may play an important role in regulating apoptotic events which may be part of the myocardial damage that occurs during acute ischemic injury. Overproduction of ROS from oxidative damage during acute ischemia and reperfusion may be one of the most important determinants involved in apoptotic death of myocardial cells [111]. However, the mechanisms by which HSPs protect cardiac myocytes against apoptosis remain to be fully elucidated, but it is believed that multiple levels in the apoptosis death cascade are involved. One proposed hypothesis is that HSP70 is able to inhibit apoptosis by preventing the release of cytochrome *c* from mitochondria through both mitochondrial-mediated pathways and receptor-mediated signaling pathways [112]. Furthermore, there is evidence suggesting that in addition to HSP70, HSP90 and low-molecular weight HSPs may provide an anti-apoptotic role by possibly acting in concert with HSP70 [95,113] (Fig. 3). Details of these proposed mechanisms have been the subject of several recent reviews [64,98,99].

1.4. Therapeutic implications

Our understanding of the cellular and molecular mechanisms involved in myocardial protection during ischemia/reperfusion injury has progressed rapidly over the past few years. Additional pathways for cellular defenses against hypoxia remain to be identified and many features of the known defense mechanisms have not been fully elucidated. Nevertheless, the plethora of knowledge currently available has provided an entry point for translating the cellular and molecular mechanistic information regarding cardioprotection and hypoxia responses into clinically relevant therapeutic or preventive strategies [114,115].

One of the most exciting strategies has been to utilize the therapeutic action of HIF transcription factors prior to or during ischemic stress as a form of preconditioning stimulus or to augment the endogenous response during ischemia. Several successful strategies have been employed in experimental animal model systems involving HIF in various ingenious and novel approaches. Transgenic mice containing constitutively active HIF-1α molecule by deletion of the central oxygen-dependent degradation domain

exhibit significantly increased activation of HIF transcriptional targets and overgrowth of blood vessels [116]. Interestingly, these vessels were not associated with increased edema and their vascular integrity appeared to be fully intact. In contrast, previous studies utilizing VEGF as a proangiogenic therapy led to leaky and nonfunctional vessels; thus, HIF activation likely provides several additional vasculogenic growth factors besides VEGF which allows for therapeutic vasculogenesis rather than inefficient angiogenesis [116]. An alternative approach has been developing a gene therapy vector containing the N-terminal DNA-binding and dimerization domain of HIF-1α fused to the strong transactivation domain of the herpes virus VP16. Administration of this naked DNA vector into the hearts of a rat myocardial infarction model resulted in an improvement in the response to hypoxia with regard to angiogenesis and reperfusion [117].

Other studies have focused on inhibiting the VHL degradation pathway of endogenous HIF-1α. Overexpression of blocking peptides against the VHL-binding prolyl hydroxylation sites in human HIF-1α has resulted in increased HIF transcriptional activity, and subsequent enhanced angiogenic responses [118]. Additionally, utilizing PR39, a macrophage-derived peptide that interacts with the proteasome and stabilizes HIF, has provided increased peri-infarct vascularization in mouse cardiac tissue [119]. However, the use of small-molecule inhibitors of the HIF hydroxylases has proven to be a most promising and exciting therapeutic pathway for preventing myocardial ischemic injury. Inhibition of the HIF hydroxylases by 2-oxoglutarate analogs stabilizes HIF, which leads to transactivation of the hypoxia response genes [19,20]. In one study, administration of a compound that inhibits EGLN1/PHD prolyl hydroxylase led to tissue preservation during myocardial infarction in rats [120].

Finally, heat shock proteins have proven to be reliable in providing myocardial protection against ROS during ischemia as well as reperfusion injury. Novel therapeutic strategies using HSPs that have been recently explored include pharmacologic interventions as well as gene transfer techniques. A previous study demonstrated that mild heat treatment, inducing expression of HSPs prior to hypothermic storage, improves the functional recovery of transplanted hearts [121]. As indicated earlier, urocortin, a member of the cardiotrophin-releasing hormone family, has been shown to protect cultured cardiomyocytes from ischemic and reperfusion injuries leading to decreased infarct size in the rat heart exposed to ischemia. It is thought that its effects may be partially mediated through the upregulation of HSP90 protein [103,107].

Furthermore, the cytoprotective hydroxylamine derivative, bimoclomol, a coinducer of heat shock proteins, especially HSP70 [122], was found to be protective in a murine model of ischemia [123]. This compound also increased the contractility of the working mammalian heart associated with increased intracellular calcium transients, and decreased the ischemia-induced depression of cardiac contractility and ST-segment elevation, as well as the occurrence of ventricular fibrillation upon reperfusion [122]. Bimoclomol elevated HSP70 in rat neonatal cardiomyocytes and protected against lethal heat stress [124]. Similarly, oral bimoclomol raised HSP70 and reduced infarct size in a rat model of ischemia and reperfusion [125]. The co-inducing effect of bimoclomol on HSP expression is mediated via the sustained activation of HSF1 via prolongation of HSF1 binding to congnate DNA elements [126]. Thus, an agent such as bimoclomol might be useful in the prevention of ischemic damage during clinical situations, such as cardiac surgery and complex vascular operations, where patients are at high risk for ischemic cardiac and other organ damage.

Recent studies also have suggested that the myocardial protection provided by angiotensin-converting enzyme inhibitors may be mediated through HSP72 and HSP73 [127]. Lastly, gene transfer techniques of *HSP* genes may present a promising strategy for therapeutic intervention in the ischemic heart. Delivery of *HSP* genes utilizing either liposome or viral vectors [76,93] may potentially be a useful strategy for increasing HSP proteins for myocardial protection.

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Symposium: Heavy Metal Exposures in Women and Children, the Role of Nutrients

Metals and Neurotoxicology[1,2]

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Abstract

Metals are ubiquitous and play a critical role in neurobiology. Transition metals are important because they alter the redox state of the physical environment. Biologically, transition metals catalyze redox reactions that are critical to cellular respiration, chemical detoxification, metabolism, and even neurotransmitter synthesis. Many metals are both nutrients and neurotoxicants, such as iron, zinc, copper, and manganese. Other metals, such as lead and cadmium, are metabolized similarly to these metals, particularly iron. Iron metabolism and genes that regulate iron metabolism may be the key to understanding metal toxicity. Finally, recent evidence demonstrates that early life exposures may program later life and adult disease phenotypes via processes of epigenetics. Parallel work in metals demonstrates that epigenetics may be a critical pathway by which metals produce health effects. J. Nutr. 137: 2809–2813, 2007.

Introduction

The biological effects of metals are linked to their chemical properties. Transition metals (such as Cu, Fe, and Mn) are particularly adept at catalyzing redox reactions within biological systems. Zn is a nutrient metal that in high dosage can paradoxically promote oxidative toxicity. Heavy metals (Pb, Cd) and metalloids (As) can also induce oxidative toxicity but more likely work by binding to proteins and interfering with metal transport and protein function. Although Pb and methylmercury neurotoxicity is well established, the effects of other metals on brain development have only recently drawn attention. Unfortunately, it appears that excess metal exposure may be a common source of neurotoxicity in multiple populations around the world.

Although metals have multiple effects on biological systems, an understudied effect is their role in programming gene expression. A growing body of evidence suggests that metals may influence epigenetic phenomena which regulate the expression of genes and ultimately their protein products. In this article, we focus on the neurotoxic properties of metals and their ability to mimic the pathways of Fe metabolism. In addition, we review the data on the effects of metals on DNA methylation and discuss how these properties might explain fetal origins of adult disease.

Neurotoxicity of Fe

Research on Fe and neurodevelopment has focused primarily on the effects of Fe deficiency anemia. Nevertheless, there is evidence that [illegible]

Several studies have noted a "U"-shaped association between maternal hemoglobin or serum ferritin (SF) and low birth weight. This association has been attributed by some investigators to a failure of normal plasma volume expansion in pregnancy (1) or to inflammation from undiagnosed perinatal infection, as serum ferritin is a well-known acute-phase reactant. Goldenberg et al. (2) showed that high maternal SF scores not only predicted low birth weight and prematurity but also predicted decreased IQ in children who were followed until age 5 y. More recently, Tamura et al. (3) demonstrated a U-shaped association between infant umbilical cord SF and lower IQ scores at age 5 y in an Alabama birth cohort. Subjects in the highest quartile of SF at birth were 3.3-fold more likely than the middle 2 quartiles (95% confidence interval: 1.2–9.1) to score below the 15th percentile in full-scale IQ. Similar findings were reported for subjects in the lowest quartile for SF, suggesting that both high and low Fe stores are associated with poor developmental outcomes. Animal studies also support these findings. Fe supplementation of rats produced a decrease in motor activity and exploratory and stereotyped behaviors similar to that of late iron deficiency (ID) anemia (4). Another report by Fredriksson et al. showed that mice administered large oral doses of Fe at postnatal d 10–12 had long-term effects on spontaneous motor behavior, with the animals showing a lack of habituation of spontaneous activity and poorer performance in the radial maze test at 3 mo of age (5).

Such findings, if validated, will undoubtedly complicate public health efforts at eradicating Fe deficiency but should not be dismissed as confounding from the effects of ferritin as an acute-phase reactant. Excess Fe is known to be neurotoxic in adults, and the possibility that it may also produce health effects in pregnant women and newborns must also be investigated.

Neurotoxicity of Mn

Unlike As and Pb, Mn is not only a toxic metal but also an essential nutrient and is required for many essential enzymatic

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reactions (6,7). Although Mn deficiencies are possible, they rarely occur in humans. The primary mechanisms of Mn neurotoxicity are not well understood but appear to involve increased oxidative damage to neuronal cells (8,9). With respect to potential developmental neurotoxicity, Tran et al. demonstrated that increased dietary Mn supplements fed to lactating dams were associated with decreased striatal dopamine levels as well as significant increases in passive avoidance errors (6,10). Excessive Mn intake from environmental and occupational settings is associated with several negative health outcomes including lethargy, tremor, and psychological and neurological disorders resembling both schizophrenia and Parkinson's disease (11). Numerous occupational studies document memory loss, anxiety, nervousness, impulsive-compulsive behaviors, psychotic experiences, fatigue, and sleep disturbances (12–14).

The main sources of environmental exposure to Mn are through the diet, inhalation, and drinking contaminated water. At least 4 studies have reported toxicity from excess Mn in children. In Chinese children, exposure to elevated Mn concentrations in drinking water were associated with lower scores on tests of short-term memory, manual dexterity, and visual-perceptual speed (15). A pilot study of children with attention deficit hyperactivity disorder demonstrated an association with this condition and higher hair levels of Mn (16). Wright et al. demonstrated an inverse association between hair Mn and IQ in grade-6 children in Oklahoma (17). Finally, Wasserman et al. (18) found associations between high water Mn levels and full-scale, performance, and verbal IQ among 142 children 10 y of age in Bangladesh.

Neurotoxicity of As

Arsenic has traditionally been classified as a peripheral neurotoxin with a clinical manifestation of polyneuropathy, but recent evidence from animal studies suggests thar As also affects the central nervous system and that prenatal exposures influence neurological phenotypes in offspring. Arsenic has been shown to pass readily through the placenta, and mice born to As-exposed dams had elevated As concentrations in their brain tissue (19). Others have shown that rats born to dams dosed orally with As had learning and behavioral deficits (20). Compared with unexposed controls, rats exposed to As prenatally had increased spontaneous locomotor activity and increased errors on memory test (20). Mechanistically, these deficits may be caused by increased oxidative toxicity. Arsenic in drinking water will produce a dose-dependent decrease in glutathione, superoxide dismutase, and catalase in the brain, indicating neurotoxic oxidative stress (21,22) Among rats exposed to As during pregnancy, fetal brain neurons underwent apoptotic changes and neuronal necrosis (23).

Historical case studies report As neurotoxicity in Japanese infants who survived an outbreak of As poisoning from contaminated milk powder in 1955 (24). Recent epidemiological studies include a cross-sectional study in Mexico that found that higher levels of urinary As were significantly related to poorer performance on verbal memory, verbal comprehension, and long-term memory (25). These findings are consistent with results from studies in Taiwan, the United States, and Bangladesh (26,18).

Neurotoxicity of Cd

The neurotoxicity of Cd in children was investigated in several studies in the 1970s and 1980s but has received little attention since. In most of these studies, the biomarker of exposure was the concentration of Cd in hair. In case-control studies in which the hair concentration of Cd of a clinically defined group was compared with that of a reference group, higher concentrations were reported in children with mental retardation (27–29), and learning difficulties or dyslexia (30,31). In cohort studies, Thatcher et al. (32,33) reported that the concentration of Cd in hair was inversely related to adjusted IQ. Other investigators (34) have reported associations between hair Cd and children's performance on visual-motor tasks. However, No population-based studies of the neurotoxicity of Cd have been conducted in children to date.

Neurotoxicity of Cu and Zn

Like Fe, most of the literature on the neurotoxicity of Cu and Zn centers around nutritional deficiency and its effect on brain (35). Also, as for Fe, there is evidence of neurotoxicity when these metals are found in excess in the brain. Cu is a transition metal, and consequently, its metabolism and toxicity are similar to those of Fe and Mn. As for Fe, genetic diseases of excess Cu retention are well described and have significant neurologic sequelae. Wilson's disease is the most common of these diseases, and the presenting complaint for this genetic disorder frequently includes neurobehavioral changes resembling schizophrenia (36). These neurologic findings may even precede other findings such as liver disease. Descriptions of environmental or excess dietary Cu producing subclinical neurobehavioral effects are very rare, but these have not been systematically studied. Excess brain Cu is a common finding in neurodegenerative diseases such as Alzheimer's disease.

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Zn, like excess Fe and Cu, is a common finding in neurodegenerative disease (37). Zn finger proteins are key transcriptional elements that regulate the cellular response to metal toxicity among other processes. Excess Zn is involved in the neuronal injury observed in cerebral ischemia, epilepsy, and brain trauma. Toxic Zn accumulation may result from either transsynaptic Zn movement or mobilization from intracellular sites, such as Zn flux through receptor-associated calcium channels, voltage-sensitive calcium channels, or Zn-sensitive membrane transporters (38). The mechanisms by which Zn exerts its neurotoxicity include mitochondrial production of reactive oxygen species and the disruption of metabolic enzymes, ultimately leading to activation of apoptotic processes. As with Cu, Fe, and Mn, an exciting new area of research is the role of Zn metabolism in Alzheimer's disease as a trigger for amyloid-β aggregation and neuronal plaque formation.

As we previously noted, excess Fe, particularly during pregnancy, has been associated with neurodevelopmental outcomes later in life, and similar studies of Cu and Zn in pregnancy are sorely needed.

Neurotoxicity of Pb and methylmercury

The literature supporting the neurodevelopmental toxicity of both Pb and methylmercury is extensive, and a comprehensive summary of either metal is beyond the scope of this article. Although controversy still exists regarding the levels at which Pb toxicity manifests itself clinically, there is widespread acceptance that Ph is neurotoxic. With respect to methylmercury, for which the primary exposure source is fish consumption, the most pressing research question at present is how to balance the beneficial effects of fish consumption vs. the toxic effects of methylmercury.

Metal mixtures and neurodevelopment

There are few studies that have reported on the effect of chemical mixtures in humans despite the fact that many metals are

commonly encountered as mixtures in the environment. Initial studies merely reported correlations among markers of internal metal dose, including a positive correlation between blood Pb and Mn (39,40), and a positive correlation between urine As and blood Pb among children (25).

With respect to neurologic outcomes, we are unaware of any clinical data investigating joint exposures to Mn and Pb. However, animal studies provide compelling evidence that exposure to both Mn and Pb lead to synergistic neurological effects. Among rats orally exposed to both Mn and Pb, motor activity and neurotransmitter levels were significantly increased, compared with rats exposed to only 1 metal (41). Exposure to Pb and Mn decreased learning of conditioned avoidance responses more than either Pb or Mn alone, and gestational exposure to both Pb and Mn reduced brain weight to a greater extent than either metal alone (42). Each of these studies also showed that coexposure to Mn and Pb led to increased brain Pb levels, perhaps because of changes in affinity of Pb-binding proteins in the brain (43). In addition, multiplicatively greater changes in monoaminergic neurotransmitter levels occur in the brains of rats exposed to Pb and As jointly, compared either metal alone (44) or to combinations of Pb, Mn, and As. Rodriguez et al. observed that Mn and As had greater accumulation in rat brains relative to controls with single metal exposures (45). The 3 metal concentrations when combined were associated decreases in dopaminergic metabolites and increases in serotinergic metabolites. Overall, these findings are complex, but the data support the concept that coexposure to multiple metals may cause neurotoxic effects not seen with exposure to a single metal at the same dose.

Fetal programming

There is growing evidence that exposure to toxicants in early life may cause later life health effects. The observed phenomenon of "fetal origins of disease" suggests that early environmental exposures, such as metals, program later life gene expression. There is an increasing search for the biological process by which programming occurs. Because DNA sequence is static, genetic susceptibility from DNA sequence variation cannot explain the mechanisms by which prenatal or early childhood metal exposures impact cognition and behavior later in life. One possible mechanistic pathway for this phenomenon, which has yet to be fully explored in humans, is epigenetics. Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. Such changes can have influences as profound as those exerted by mutations but, unlike mutations, are reversible and responsive to environmental influences. DNA methylation is the best studied of the epigenetic processes that regulate gene silencing. In general, increased methylation is inversely associated with gene expression. DNA methylation has been associated with chromosome packaging and heterochromatin formation and determines the 3-dimensional space through which transcription factors can or cannot attach to the DNA sequence. Specifically, the methylated cytosine in DNA promoter regions serves as a "mutation" of a promoter region recognition element, functionally reducing the binding affinity of the response element for its transcription factor. Although in the strictest sense epigenetics refers to changes in germ cell DNA methylation, the process of DNA methylation is critical more globally to cell differentiation and overall child development. Failure of DNA methylation systems in the brain leads to clinical syndromes such as mental retardation and autistic-like behaviors (46). Animal studies increasingly demonstrate that environmental factors can alter DNA methylation patterns and that these changes correlate with animal behavior (47).

The growing interest in epigenetic markers is a result of their potential to explain fetal origins of disease or even simply explain the latency between exposure to toxic substances and subsequent disease phenotypes. Although DNA methylation patterns in different tissues are largely constitutive, DNA methylation patterns are still subject to active regulation in the nervous system in response to environmental stimuli. Endres et al. (48) recently demonstrated that levels of DNA methylation activity in the brain are increased with ischemic injury. Others have shown that diet can impact methylation and behavior. For example, L-methionine treatment can exacerbate psychosis, whereas valproate, a drug producing hypomethylated DNA, reduces such symptoms (49,50). Epigenetic modifications of regulatory DNA sequences in response to subtle variations in environmental conditions might be a critical source of variation in gene expression and function. If so, DNA methylation changes may serve as a process mediating the relationship between genome and environment throughout neurodevelopment. The effects of prenaral/early metal exposure on DNA methylation may program environmental exposures on the fixed genome, resulting in subtle but stable alterations in later life neurophenotypes.

Metals and DNA methylation

Several studies have established an association between DNA methylation and environmental metals, including Ni, Cd, Pb, and particularly As (51–53). Oxidative stress may be a unifying process to explain these findings across different metals. Metals are known to increase reactive oxygen species production in a catalytic fashion via redox cycling (54,55). Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA (56), thus resulting in a generalized hypomethylation of cytosine residues at CpG sites (57). In addition, Takiguchi et al. (58) showed that Cd inhibited DNA methyltransferases in a manner that was noncompetitive with respect to the DNA substrate. This finding is suggestive of interference in enzyme-DNA interaction, possibly through an interaction of Cd with the methyltransferase DNA binding domain (58).

As is the best-studied metal with effects on DNA methylation. Several in vitro studies have shown that As is associated with global DNA hypomethylation (59–61) as well as gene-specific DNA hypermethylation (62,63). This effect might be explained by the overlap between As metabolism and DNA methylation processes. Both consume S-adenosylmethionine, the universal methyl donor, which is a critical cofactor for both DNA methylation and the methylation of xenobiotics. In animal models, global DNA hypomethylation induced during gestation has been shown to perturb the function and survival of central nervous system neurons (64). Metal-induced alterations in methylation metabolism could initiate a cascade of events including gene-specific DNA hypo- or hypermethylation, resulting in aberrant gene expression and also in diminished glutathione activity leaving cells more vulnerable to oxidative stress. Although the results of these epigenetic changes on neurodevelopment have remained unexplored, given the clear importance of DNA methylation to processes of neurodevelopment, the metal-induced disruption of DNA methylation clearly deserves further study.

Neurotoxicity is a common health endpoint for excess metal exposure. Even nutritional metals, such as Fe and Mn, are neurotoxic in excess. Because real-life scenarios include exposures to multiple metals simultaneously, there is a growing need for research on mixtures of metals and their health impact. Finally, there is intriguing evidence that epigenetic phenomena may underlie observed effects of fetal or early life exposure and late onset of disease. Metals appear to alter DNA methylation, an epigenetic

process by which gene expression is regulated. Further research in metals should include the role of epigenetics in determining long-term and late-onset health effects from metal exposure.

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Recovery of precious metals through biosorption — A review

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ABSTRACT

Recovery of precious metals like gold, silver, palladium platinum etc. is interesting due to its high market prices along with various industrial applications. Conventional technologies viz. ion exchange, chemical binding, surface precipitation etc. which been have been developed for the recovery of such metals are not economically attractive. Biosorption represents a biotechnological innovation as well as a cost effective excellent tool for recovery of precious metals from aqueous solutions. A variety of biomaterials are known to bind the precious metals including algae, fungi, bacteria actinomycetes, yeast etc. along with some biopolymers and biowaste materials.The metal binding mechanism , as well as the parameters influencing the uptake of precious metals and isotherm modeling are presented. This article provides an overview of past achievements and present scenario of biosorption studies carried out on the use of some promising biosorbents which could serve as an economical means for recovering precious metals. The present review also highlights the use of biosorbents in real situations and hopes to provide insights into this research frontier.

1. Introduction

Precious metals are widely used as catalyst in various industries, agriculture and medicine, because of their specific physical and chemical properties (Ramesh et al., 2008). Economically, the precious metals are historically important as currency, and remain important as investment commodities. Gold (Au), silver (Ag), platinum (Pt) and palladium (Pd) are internationally recognized as forms of currency under ISO 4217. Because of the limited availability of the precious metals, recovery of these metals from aqueous and waste solutions is economically attractive. Industrial cycling techniques such as pyro-metallurgical and hydrometallurgical processes have been widely used to recover precious metals from wastewater (Jacobsen, 2005). The hydrometallurgical methods, which includes adsorption by ion exchange resin, solvent extraction, and the reduction of precious metal precipitate by reagents, has been utilized more often than the pyro-metallurgical process. Both of these recovery methods are costly and require extensive labour and time. Furthermore, large quantities of secondary waste are generated resulting from the addition of chemical agents for precipitation and reduction in the processes. Therefore, there is a need to develop a system to recover precious metals from wastewater i.e. low cost and eco-friendly.

Biosorption is considered promising technology for the recovery of precious metals. This is due to the fact that the properties of certain types of inactive or dead microbial biomass materials allow them to bind and concentrate metal ions from industrial effluents and aqueous solutions (Dobson and Burgess, 2007). These types of biomasses are relatively inexpensive and available in large quantities. Biosorption is a metabolism independent process that takes place in the cell wall (Mao et al., 2009), and the mechanism responsible for the metal uptake may differ according to the biomass type.

Cost effectiveness is the main attraction of metal biosorption. Since biosorption often employs dead biomass, this eliminates the need of nutrient requirement and can be exposed to environments of high toxicity (Volesky, 1990). A major advantage of biosorption is that it can be used in situ, and with proper design may not need any industrial process operations and can be integrated with many systems in the most eco-friendly manner (Tewari et al., 2005).

In the present article, the use of different microorganisms including bacteria, fungi, algae, actinomycetes, yeasts and some biowaste materials on precious metal biosorption have been reported. Recently, biopolymers have also received a great deal of attention for serving as potential biosorbent of precious metals.

2. Overview of treatment methods

Various methods have been employed for the recovery of precious metals. Methods which have been applied to the recovery of gold from its solution include zinc dust cementation (Wan and Miller, 1990; Miller et al., 1990), carbon adsorption (Xu et al., 1995), electrodeposition (Wan and Miller, 1986) , solvent extraction (Mooiman and Miller, 1991) and ion exchange (Gomes et al., 2001). The main technologies used for silver removal from wastewaters include

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precipitation, ion exchange, reductive exchange and electrolytic recovery (Lambert and Murr, 1966; Cotton and Ikinson, 1972). For the platinum group metals (PGM), solvent extraction (Barnes and Edwards, 1982) is a traditionally used method for PGM recovery. Alternatively, ion exchange (Els et al., 2000; Iglesias et al., 1999; Rovira and Hurtado, 1998), membrane separation (Takahiko et al., 1996), and adsorption (Liu et al., 2000) have been developed for the recovery of PGM from solution. Comparatively, the adsorption seems to be the most suitable method for the recovery of PGM in the case of low concentration due to low cost and high efficiency (Veglio and Beolchini, 1997).

In recent years, research attention has been focused on biosorption, a cost effective biological method which has been demonstrated to possess good potential to replace conventional methods for recovery of precious metals using various biosorbents.

3. Biosorbents

3.1. Biosorbents of gold

The demand for gold (Au) has been increasing since it is used as a momentary standard and its wide utilization in different applications. Gold is commonly used in electronics, jewelry, and anti arthritis drugs (Pethkar and Paknikar, 1998; Shaw, 1999).The increased demand for gold has increased interest in the recovery of gold from waste solutions such as those from refining or mining effluents with high concentrations of gold.

Some researchers have investigated the recovery of gold using biosorbents such as algae (Hosea et al., 1986; Kuyucak and Volesky, 1989), fungi (Gomes et al., 1998; Matsumoto and Nishimura, 1992; Pethkar and Paknikar, 1998) and yeasts (Karamuchka and Gadd, 1999). Activated carbon has traditionally been used to adsorb gold from aqueous solution. However, its manufacturing and regenerating poses several constraints and represents a major portion of the operating cost in a mining operation (Kuyucak and Volesky, 1986). Furthermore, the elution of gold from activated carbon requires the use of high temperatures and the use of special kilns to reactivate the carbon (Staker and Sandberg, 1987). An alternative to the use of activated carbon could involve the use of cost effective biosorbents for the recovery of gold ions from aqueous solutions.

Algae have been proved to be efficient and economical biosorbent for the recovery of gold from aqueous solution. Au (III) was successfully recovered as metallic gold nanoparticle using dead biomass of the brown alga, *Fucus vesiculosus* (Mata et al., 2009). *Chlorella vulgaris*, a green algae was capable of removing more than 90% of the gold from very dilute solution (Hosea et al., 1986; Ting et al., 1995). The brown marine alga *Sargassum natans* was also found to be highly selective for gold (Kuyucak and Volesky, 1990).

The fungal cells of *Aspergillus niger*, *Mucor rouxii* and *Rhizopus arrihus* were found to take up precious metals like gold (Mullen et al., 1992; Townsley and Ross, 1986). Two strains of a fungus, *Cladosporioum cladosporoides* 1 and C. *cladosporoides* 2 showed preferential sorption of gold (Pethkar et al., 2001). The fruiting body of a bracket fungi (*Fomitopsis carnea*) immobilized in polyvinyl alcohol was used as biosorbent and the gold (III) recovery was found to be 94 mg/g (Khoo and Ting, 2001). Out of 17 different fungal strains, *A.oryzae*, *Chaetomium globosum*, *Giberella fujikuroi*, *Mucor hiemalis*,*Penicilium chrysogenum* and *P. lilacinum* showed good biosorption capacity of gold (Tsuruta, 2004).

Among the 19 actinomycetes strains, *Strptomyces phaeochromogenes* HUT6013 showed maximum biosorption of gold (282 μmol/g dry wt cells). Gold biosorption capacities of 14 strains of different yeast species viz. *Candida krusei*, *C. robusta*, *C. utilis*, *Cryptococcus albidus*, *C. laurentii* Debaromyces *hansenii*, *Endomycopsis fibigera*, *Hansenula anomala*, *H. saturnas*, *Kluyveromyces Pichia farinose*, *Saccharomyces cerevisiae*, *Sporobolomyces salmonicolor* and *Torulopsis aeria* were also reported (Tsuruta, 2004). Au^3+ biosorption by waste biomass of *Saccharomyces cerevisiae* have been reported by Lin et al. (2005a)). The yeast biomass showed remarkable affinity for the gold ions due to its oxygenous functional groups on the cell wall.

High abilities of gold biosorption by gram negative bacterial strains viz. *Acinetobacter calcoaceticus*, *Erwinia herbicola*, *Pseudomonas aeruginosa* and *P. maltophilia* from a solution containing hydrogen tetrachloroaurate have been reported by Tsuruta (2004). Magnetotactic bacteria (MTB) have been investigated by Huiping et al. (2007) as biosorbent for the adsorption of Au (III) from aqueous solution.

The animal product, hen egg shell membrane was evaluated for its ability to recover gold ions, Au(I) and Au(III) from solutions and electroplating wastewater (Ishikawa et al., 2002).

The plant biomass like alfalfa biomass which was able to reduce gold (III) to Gold (0) was reported by Gardea-Torresdey et al. (2002). Gold (III) recovery from multi-elemental solutions was also reported by Gamez et al. (2003). Chemically modified hop biomass was evaluated for binding and reduction of Au (III) by López et al. (2005). The use of persimmon peel gel for the recovery of Au (III) from aqueous chloride medium was investigated by Parajuli et al. (2007).

Recently, selective adsorption of gold by discarded agro waste materials viz. rice husk carbon and barley straw carbon has been reported by Chand et al. (2009).

Biopolymer, chitosan is a deacetylated derivative of chitin, the second most abundant biopolymer on earth after cellulose. The major advantage of using chitosan for precious metal sorption is that the amino sites of chitosan are easily protonated in acid media, accentuating the electrostatic forces often implicated in the initial stages of sorption. Chitosan can be easily modified by grafting new functional groups onto the polymer backbone to increase its range of properties and functionalities (Mack et al., 2007). Glutaraldehyde-crosslinked and sulfer grafted chitosan were reported as sorbent of Au (III) by Arrascue et al. (2003). Chemically modified chitosan was used and uptake values of gold was reported as 3.4 mmol/g (Donia et al., 2007). Crosslinked chitosan resin chemically modified with l-lysine was used to investigate the adsorption of Au (III) from aqueous solutions (Fujiwara et al., 2007). The maximum adsorption capacity was found to be 70.34 mg/g for Au (III).

Activated hard shell of Iranian apricot stones has been proved as a promising adsorbent for gold recovery (Soleimani and Kaghazchi, 2008). The results showed that under the optimum operating conditions more than 98% of gold ions were adsorbed onto activated carbon just after 3 h.

Tasdelen et al. (2009) demonstrated that gold recovery using DEAE-cellulose was effective for recovering gold from diluted gold chloride solutions. Using excessive amounts of DEAE-cellulose (at DEAE-cellulose/Au weight ratios of 400 and above), gold recovery efficiency was found to be over 99%.

Table 1 summarizes the different types of adsorbents used for gold biosorption.

3.2. Biosorbents of silver

Silver is a noble metal that has been widely employed in the photographic and imaging industry for many years. It is known to be released to the environment through its industrial applications, leading to the possible exposure of aquatic organisms (Pedroso et al., 2007). Most world silver is recovered from scrapes such as photographic fims, X ray films and jewelry (Ajiwe and Anyadiegwu, 2000).

Silver binding properties of two strains of alga *Chlorella vulgaris* (211/11b and 211/12) was investigated by Cordery et al. (1994). Rapid Ag^+ uptake from aqueous solution was proved. Biosorption of silver ions by processed *Aspergillus niger* biomass was reported by Akthar et al. (1995).

Ag^+ biosorption by an industrial strain of *Saccharomyces cerevisiae* was investigated by Simmons and Singleton (1996). Older (96 h old)

Table 1
Biosorption of gold (Au^{3+}) using different adsorbents.

Adsorbents	pH	Q_{max} (mmol/g)	Reference
Algae			
Fucus vesiculosus	7.0	0.35	Mata et al. (2009)
Dealginated Seaweed Waste	3.0	0.4	Romero-Gonzalez et al. (2003)
Sargassum fluitans	2.0	0.0032	Niu and Volesky (1999)
Chlorella vulgaris	6-7	0.5	Cordery et al. (1994)
Sargassum natanss	2.5	2.1	Kuyucak and Volesky (1988)
Ascophyllum nodosum	2.5	0.15	Kuyucak and Volesky (1988)
Chlorella vulgaris	2.0	0.5	Darnall et al. (1986)
Fungi			
C. cladosporioides Strain 1	4.0	0.4	Pethkar et al. (2001)
C. cladosporioides Strain 2	4.0	0.5	Pethkar et al. (2001)
Fomitopsis carnea	1-13	0.48	Khoo and Ting (2001)
PVA-immobilized biomass			
Saccharomyces cerevisiae	5.0	0.026	Savvaidis (1998)
Cladosporium cladosporioides	4.0	0.5	Pethkar and Paknikar (1998)
Cladosporium cladosporiaides	4.0	0.18	Pethkar and Paknikar (1998)
Aspergillus niger	2.5	1.0	Kuyucak and Volesky (1988)
Rhizopus orrhizus	2.5	0.8	Kuyucak and Volesky (1988)
Bacteria			
Streptomyces erythraeus	4.0	0.03	Savvaidis (1998)
Spirulina platensis	4.0	0.026	Savvaidis (1998)
Others			
Rice husk carbon	-	0.76	Chand et al. (2009)
Chemically modified chitosan	-	3.4	Donia et al. (2007)
Crosslinked chitosan resin chemically modified with l-lysin	-	70.34 mg/g	Fujiwara et al. (2007)
Bisthiourea derivative of resins	2.0	3.63	Atia (2005)
Condensed-tannin gel	2.0	40.0	Ogata and Nakano (2005)
Alfalfa	5.0	0.18	Gamez et al. (2003)
Sulfur derivative of chitosan (RADC)	3.2	3.2	Arrascue et al. (2003)
Glutaraldehyde crosslinked chitosan (GCC)	1.6	2.9	Arrascue et al. (2003)
Hen eggshell membrane (ESM)	3.0	0.67	Ishikawa et al. (2002)
Dealginated Seaweed Waste	3.0	0.4	Romero-Gonzalez et al. (2003)

biomass had half the biosorption capacity of younger (24 h old) biomass (0.187 and 0.387 mmol Ag^{+} /g dry mass respectively). Incorporation of l-lysine from 0 to 5.0 mM into the growth medium increased silver biosorption from 0.389 to 0.556 mmol Ag^{+}/ g dry biomass.

Bacterial strains viz. BP 7126 (a *Pseudomonas* sp. strain isolated from a silver mine and in sediments of the river Inn in Austria), ER 121 (a slime producing alkaligenes eutrophus strain, isolated from soil contaminated by a non-ferrous industrial plant in Belgium) and AS 302; *Pseudomonas mendocinu* strain, isolated from a copper mine in Likasi South in Zaire) showed the potentiality of silver biosorption (Tsezos et al., 1997). Silver biosorption by *Lactobacillus* sp strain A09 was reported by Lin et al. (2005b).

The uptake behavior of various resins towards Ag (I) adsorption from aqueous medium was investigated by Atia (2005b). Recycling of silver from processed photo film was also carried out and the efficiency was compared with that of a commercial resin.

Donia et al. (2007) reported the recovery of silver from aqueous solution using chemically modified chitosan resin with magnetic properties and uptake values of silver was found to be 2.1 mmol/g. Table 2 summarizes the different types of adsorbents used for silver biosorption.

3.3. Biosorbents of platinum (Pt) and palladium (Pd)

Platinum group metals (PGM) viz. platinum and palladium are precious metals and are widely used in industries because of their specific physical and chemical properties. The effective recovery of PGM from both natural ore and industrial waste is quite important from the viewpoint of full utilization of resources.

Table 2
Biosorption of silver using different adsorbents.

Adsorbents	pH	Q_{max} (mmol/g)	Reference
Fungi			
C. cladosporioides Strain 1	4.0	0.6	Pethkar et al. (2001)
C. cladosporioides Strain 2	4.0	0.12	Pethkar et al. (2001)
Aspergillus niger	5-7	0.9	Akthar et al. (1995)
Neurospora crassa	5-7	0.6	Akthar et al. (1995)
Fusarium oxysporium	5-7	0.5	Akthar et al. (1995)
Yeast			
Saccharomyces cerevisiae	-	0.387	Simmons and Singleton (1996)
Others			
Chemically modified chitosan resin	-	2.1	Donia et al. (2007)
Bisthiourea derivative of resins	6.5	8.25	Atia (2005b)

Guibal et al. (1999b) studied the recovery of platinum ions from dilute solutions by chitosan cross linked with glutaraldehyde. Godlewska -Zylkiewicz and Kozlowska (2005) reported the binding of Pt and Pd by *Saccharomyces cerevisiae*. Immobilised *Chlorella vulgaris* has also been studied as a Pt and Pd biosorbent (Dziwulska et al., 2004). The adsorption of Pt (IV) and Pd (II) on bayberry tannin immobilized collagen fiber (BTICF) membrane was investigated by Ma et al. (2006). The adsorption capacities of Pt (IV) and Pd (II) on 0.100 g BTICF membrane were 41.7 and 27.5 mg/g at 313 K. Crosslinked chitosan resin chemically modified with l-lysine has been used to investigate the adsorption of Pt (IV) and Pd (II) from aqueous solutions (Fujiwara et al., 2007). The maximum adsorption capacity was found to be 129.26 mg/g for Pt (IV) and 109.47 mg/g for Pd (II).

Recovery of platinum by a high performance biosorbent, polyethylenimine (PEI)-modified biomass, prepared by attaching PEI onto the surface of inactive *Escherichia coli* biomass was reported by Won et al. (2010). Wastewater collected from an industrial laboratory for inductively coupled plasma (ICP) using PEI modified biomass was studied. The maximum platinum uptake of PEI-modified biomass was enhanced up to 108.8 mg/g compared to 21.4 mg/g of the raw biomass (Won et al. (2010)).

Recently, the biosorption potential of *Racomitrium lanuginosum* as aquatic moss biosorbent for the removal of Pd (II) from aqueous solution has been reported (Sari et al., 2009). The adsorption capacity of barley straw carbon for Pt (IV) and Pd (II) has been found to be 0.39 and 0.64 mmol /kg (Chand et al., 2009).

Tables 3 and 4 summarize the different types of adsorbents used for platinum and palladium biosorption.

4. Biosorption experimental procedures

A biosorption process can be performed via several modes; of which, batch and continuous modes of operation are frequently employed to conduct laboratory scale biosorption processes. Although most industrial applications prefer a continuous mode operation, batch experiments

Table 3
Biosorption of platinum (Pt^{4+}) using different adsorbents.

Adsorbents	pH	Q_{max} (mmol/g)	Reference
Bacteria			
Desulfovibrio desulfuricans	2.0	0.32	de Vargas et al. (2004)
Desulfovibrio fructosivorans	2.0	0.17	de Vargas et al. (2004)
Desulfovibrio vulgaris	2.0	0.17	de Vargas et al. (2004)
Others			
Bayberry tannin immobilized Collagen fiber (BTICF) membrane	3.0	0.23	Ma et al. (2006)
Thiourea derivative of chitosan	2.0	2.0	Guibal et al. (1999a,b)
Glutaraldehyde crosslinked chitosan	2.0	1.6	Guibal et al. (1999a,b)
Chitosan derivatives	2.0	3.2	Chassary et al. (2005)

Table 4
Biosorption of palladium (Pd^{2+}) using different adsorbents.

Adsorbents	pH	Q_{max} (mmol/g)	Reference
Bacteria			
Desulfovibrio desulfuricans	2.0	1.2	de Vargas et al. (2004)
Desulfovibrio fructosivorans	2.0	1.0	de Vargas et al. (2004)
Others			
Aquatic moss *(Racomitrium lanuginosum)*	5.0	37.2 mg/g	Sari et al. (2009)
Bayberry tannin immobilized Collagen fiber (BTICF) membrane	4.0	0.32	Ma et al. (2006)
Chitosan derivatives	2.0	3.5	Chassary et al. (2005)
Rubeanic acid derivative of chitosan	2.0	3.24	Guibal et al. (2002)
Thiourea derivative of chitosan	2.0	2.54	Guibal et al. (2002)
Glutaraldehyde crosslinked chitosan	2.0	2.44	Guibal et al. (2002)

have to be used to evaluate the required fundamental information, such as biosorbent efficiency, optimum experimental conditions, biosorption rate and possibility of biomass regeneration.

5. Factors influencing batch biosorption of precious metals

Batch experiments usually focus on the study of factors influencing biosorption, which are important in the evaluation of full biosorption potential of any biomaterial. The important factors include: i) solution pH ii) temperature iii) biomass dosage iv) ionic strength v) initial solute concentration and vi) agitation rate and period.

5.1. Solution pH

The solution pH is one of the most important variables which affect the speciation of metals in solution through hydrolysis, complexation and redox reactions during metal recovery (Esposito et al., 2002). This factor is capable of influencing not only the binding site dissociation state, but also the solution chemistry of the target metal in terms of hydrolysis, complexation by organic and or/ inorganic ligands and redox potentials (Fiol et al., 2006).

Gold is routinely present in solution in anionic form. Sakaguchi et al. (1995) reported that sorption of gold showed a maximum value at pH 3 using immobilized persimmon tannin. Maximum biosorption of gold (80%) occurred under acidic pH (pH 1–5) using fungal isolate *Cladosporium cladosporoides* (Pethkar and Paknikar, 1998). Uptake of gold by an inactivated alfalfa biomass was slightly higher at pH 2, but practically pH independent between pH 2 and 6 (Gardea-Torresdey et al., 1999). Effect of pH on gold uptake was examined in relation to gold speciation and the surface charge of the fungal biosorbent. Maximum uptake occurred at pH 3.9. An analysis of the zeta potential profile of the biosorbent together with gold speciation was effectively employed to explain the effect of pH on biosorption. Charge reversal of the biosorbent which occurred at pH 1.85 accounted for the decline in uptake below pH 2.0, and the formation of hydroxide chloride complexes of gold above pH 4 appeared to be responsible for reduced uptake (Ting and Mittal, 2002).

The optimum gold uptake by animal by product, hen egg shell membrane was found to be at pH 3.0 (Ishikawa et al., 2002). Gold biosorption using four different derivatives of chitosan: GCC, CHMDI, RADC and RADC-HMD were studied by Arrascue et al. (2003). The optimum pH appeared to be around pH 2–3, depending on the sorbent.

Hop biomass was reported to be capable of adsorbing significant amounts of Au (III) from aqueous solutions (López et al., 2005). Batch studies showed that Au (III) binding was fast, occurring within the first 5 min of contact and in a pH dependent manner. However, esterified hop biomass behaved in a pH independent manner and the binding was found not to change with changing pH.

Magnetotactic bacteria (MTB) as biosorbents for the adsorption of Au (II1) ions from aqueous solution have been investigated (Huiping et al., 2007). The optimum pH condition was noted 1–5.5 for Au (III).

The effect of the pH of the medium on the uptake of Au (III) was investigated by Donia et al. (2007) using chemically modified chitosan. It was found that the uptake of Au (III) slightly increased as the pH increased in the pH range 0.5 to 2.0. Since the uptake process takes place in a strong acid medium, the dominant mechanism of interaction is probably due to salt formation ($R{-}CH{=}NH + AuCl_4^-$). This interaction mechanism may take place along with adduct formation ($R{-}S \rightarrow AuCl_4^-$) due to the strong oxidizing power of $AuCl_4$-species (Donia et al., 2005).

The effect of pH on the gold uptake with *Fucus vesiculosus* from $AuCl_4^-$ solution was studied in the pH range of 2–11. The recovery and reduction process took place in two stages with an optimum range of 4-9 with a maximum uptake obtained at pH 7 (Mata et al., 2009).

Silver is normally present in the solution in cationic form. The effect of the pH of the medium on the uptake of Ag (I) was investigated by Donia et al. (2007) using chemically modified chitosan. For Ag (I), the uptake increased as the pH increased till reaching a maximum around pH 6.This behavior indicated the complex formation mechanism between Ag (I) and the donor atoms (N/S) on the resin (Atia et al., 2005, 2006).

The effect of pH on the binding capacity of Ag + to *Lactobacillus sp.* strain A09 was studied by Lin et al. (2005b). It was found that binding rate constants were pH dependent with maximum rate constant and minimum half life period occurred near pH 5.0.

For selective binding of platinum and palladium, acidic pH (0.5–4.5) was found to be effective using alga *Cholrella vulgaris*, immobilized on Cellex- T (Dziwulska et al., 2004). Retention of platinum was most efficient in the pH range of 1.5–2.0. The same range was found for the yeast *Saccharomyces cerevisiae* immobilized in calcium alginate (Godlewska -Zylkiewicz and Kozlowska, 2005) where palladium absorption was most efficient at pH in the range 1.6–2.2 and decreased with the decrease in solution acidity. Parsons et al. (2003) reported that pH 3 was optimum for platinum binding on alfalfa biomass. Chemical modification of alfalfa biomass resulted in an increase in the pH and maximum platinum binding occurred at pH 6.

5.2. Temperature

Temperature of the solution is important factor during the process of biosorption (Panda et al., 2006) though different scenario was presented by many workers. According to Aksu et al. (1992), temperature seems not to influence the biosorption performances in the range of 20–35 °C. Bhainsa and D' Souza (1999) also reported many reactions to be temperature independent or exothermic (Sag and Kutsal, 2000). A significant temperature effect on Gold (III) uptake by the egg shell membrane material was observed at 65 °C (Ishikawa et al., 2002).

The effect of temperature (30-50 °C) on the sorption of platinum by immobilized bayberry tannin was studied by Wang et al. (2005). Platinum and palladium adsorption capacity increased with the increase in temperature. Maximum specific uptake (Q_{max}) was found to be increased with an increase in temperature at 50 °C of 0.599 mmol Pt (II)/g and 1.29 mmol Pd (II) /g respectively.

Higher temperature resulted in higher adsorption extent, especially when a smaller amount of bayberry tannin immobilized collagen fiber (BTICF) membrane dose was used. For example, when temperature was raised from 293 to 313 K, the adsorption extent increased from 68.4% to 92.8% for Pt (IV) and from 44.2% to 71.1% for Pd (II) with 0.100 g membrane (Ma et al., 2006).

Fujiwara et al. (2007) studied the effect of temperature on adsorption of Pt (IV), Pd (II) and Au (III) ions onto l-lysine modified crosslinked chitosan resin by LMCCR using 100 mg/l initial metal concentration at 30, 40 and 50 °C. The results showed that the adsorption capacity decreased with increasing temperature which indicated that the adsorption process was exothermic in nature.

In case of magnetotactic bacteria (MTB), Huiping et al. (2007) reported that an increase of temperature in the interval of 15-20 °C dealt with an increase in the adsorption yield of Au(II) and Cu(II) and began to decrease slowly with increasing temperature from 25 to 35 °C. As a whole, both metal ions were effectively adsorbed by MTB in the range of 10–35 °C.

5.3. Biosorbent dosage

The dosage of a biosorbent strongly influences the extent of biosorption. An increase in the biomass concentration generally increases the amount of solute biosorbed. Due to increased surface area of the biosorbent which in turn increases the number of binding sites (Esposito et al., 2001). On the other hand, the quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent dosage which may be due to complex interaction of several factors. An important factor at high sorbent dosages is that the available solute is insufficient to completely cover the available exchangeable sites on the biosorbent, usually resulting in low solute uptake (Tangaromsuk et al., 2002). Also, as suggested by Gadd et al. (1988), the interference between binding sites due to increased biosorbent dosages cannot be overruled, as this will result in a low specific uptake.

The effects of bayberry tannin immobilized collagen fiber (BTICF) membrane dosage on the adsorption extent of Pt (IV) and Pd (II) were studied by Ma et al. (2006). The amount of membrane significantly influenced the adsorption extent of the two metal ions. At 293 K, the adsorption extents of Pt (IV) and Pd (II) were 68.4% and 44.2% by using 0.100 g membrane, while they were increased to 97.4% and 95.0% with 0.400 g membrane.

Huiping et al. (2007) found that the biosorption capacity of gold (III) or Cu(II) ions on magnetotactic bacteria decreased with an increase of biomass concentration(2.0–12.0 $g.L^{-}$,wet-mass basis).The maximum adsorption yield was achieved at biomass concentrations (wet mass basis) of more than 10.0 $g.L^{-}$.

5.4. Ionic strength

Another important parameter in biosorption is the ionic strength, which influences the adsorption of solute to the biomass surface (Borrok and Fein, 2005). The effect of ionic strength may be ascribed to the competition between ions, changes in the metal activity, or in the properties of electrical double layer. When two phases biomass surface and solute in aqueous solution are in contact, they are bound to be surrounded by an electrical double layer owing to electrostatic interaction. Thus adsorption decreases with increase in ionic strength (Donmez and Aksu, 2002). Some inorganic ions, such as chloride, may form complexes with some metal ions and therefore, affect the sorption process (Borowitzka, 1988). In high chloride environments, complication arises from the presence of a large excess of chloride ions which cause strong competition between the anions of the acid (chloride) and the metal species restricting the metal sorption in industrial waste water environment (Godlewska -Zylkiewicz, 2003). This chloride competition was reported by Dziwulska et al. (2004) who used immobilized *Chlorella vulgaris* as a biosorbent of platinum and palladium.

The effect of co-ions viz. Cl^{-}, Br^{-}, I^{-} and CN^{-} anions in the solution on the gold uptake capacities of egg shell membrane (ESM) was studied by Ishikawa et al (2002). The gold uptake capacity of ESM was depressed in the order of $Cl^{-}<Br^{-}<I^{-}<CN^{-}$ increasing the concentration of co ions from 0.01 to 0.1 mol /l.

5.5. Initial solute concentration

Initial metal ion concentration seems to have impact on biosorption, with a higher concentration resulting in a high solute uptake (Ho and McKay, 2000; Binupriya et al., 2007). This is because at lower initial solute concentrations, the ratio of the initial moles of solute to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial metal concentration. However, at higher concentrations, the sites available for sorption become fewer compared to moles of solute present; hence, the removal of solute is strongly dependent upon the initial solute concentration. Experimental studies were carried out by Fujiwara et al. (2007) using l-lysine modified crosslinked chitosan resin (LMCCR) as biosorbent with varying initial metal ion concentrations of Pt(IV), Pd(II) and Au(III), ranging from 20 to 200 mg/l using 3.33 g/l of adsorbent dose at pH 1.0 for Pt(IV), at pH 2.0 for Au(III) and Pd(II) . The results demonstrated that the amount of Pt (IV), Pd (II) and Au (III) adsorbed increased with increasing metal ion initial concentration and also the adsorption percentage decreased with increasing initial metal ion concentration. This is due to the total available sites are limited at fixed adsorbent dose, thereby adsorbing almost the same amount of solute, resulting in a decreasing of percentage adsorption corresponding to an increase in initial metal ion concentration.

5.6. Agitation rate and period

The rate of biosorption process can be influenced by external film diffusion. With appropriate agitation, this mass transfer resistance can be minimized. When increasing the agitation rate, the diffusion rate of a solute from the bulk liquid to the liquid boundary layer surrounding particles becomes high due to enhanced turbulence and the decrease in thickness of the liquid boundary layer (Evans et al., 2002). Under these conditions, the value of the external diffusion coefficient becomes larger (Shen and Duvnjak, 2005).Finally, at higher agitation rates, the boundary layer becomes thin, which usually enhances the rate at which a solute diffuse through boundary layer.

The gold recovery efficiency using DEAE cellulose was investigated as a function of agitation rate in the range of 20 to 140 rpm (Tasdelen et al., 2009).The duration of the experiments was limited to 30 min. It was evident that speeds in the range of 120–140 rpm were optimal and increasing of shaking rate resulted an increase in the gold recovery efficiency for all quantities. By increasing the shaking rate from 20 to 120 rpm, the gold recovery efficiency increased by ca 50%, which indicated that shaking was necessary for gold recovery from diluted gold-bearing solutions. In this experimental series, the effect of reaction time on gold recovery efficiency was studied in the range of 0.5–2 h. It was demonstrated that increasing the reaction time had a positive effect on gold recovery efficiency, i.e., the gold recovery efficiency increased with increased time. This finding was also in accordance with the scientific literature (Ogata and Nakano, 2005). However, in some cases, it was reported that allowing further reaction after a certain time leads to a decrease in the recovery efficiency due to back dissolution of the metal into the solution (Tasdelen et al., 2009).

The effect of agitation period on the adsorption of Pt (IV), Pd (II) and Au (III) by LMCCR was studied by Fujiwara et al. (2007). The results demonstrated that the adsorption increased with increase in agitation time and attained the equilibrium at around 120 min for Pt (IV), Pd (II) and Au (III). Over75% adsorption was occurred within 30 min and the equilibrium was attained within 120 min.

6. Biosorption equilibrium models

Models play an important role in technology transfer from laboratory to industrial scale. Appropriate models can be helpful in understanding the process mechanisms, analyzing experimental data, predicting answers to operational conditions and process optimization (Volesky and Holan, 1995; Limousin et al., 2007).

Preliminary testing of solid–liquid absorption system is based on two types of investigations: a) equilibrium batch sorption tests and b)

dynamic continuous flow sorption studies. The equilibrium of the biosorption process is often described by fitting the experimental points with models usually used for the representation of isotherm adsorption equilibrium (Gadd et al., 1998). Different types of adsorption isotherm models viz. Freundlich model (Freundlich, 1906); Langmuir model (Langmuir, 1918); Redlich and Peterson (1958); Sips model (Sips, 1948); Toth model (Toth, 1971); Radke-Prausnitz, model (Radke and Prausnitz, 1972) etc. have been used to quantify and contrast the performance of different biosorbents.

The two most widely used adsorption isotherms are the Langmuir and Freundlich isotherms. The Langmuir isotherm assumes a surface with homogeneous binding sites, equivalent sorption energies, and no interaction between sorbed species. The Freundlich isotherm is an empirical equation based on an exponential distribution of sorption sites and energies. These linearised equilibrium adsorption isotherm models for single solute system are given by the following equations: Langmuir:

$$q = \frac{q_{max}\, b\, C_{eq}}{1 + b\, C_{eq}}$$

where q is milligrams of metal accumulated per gram of the biosorbent material; C_{eq} is the metal residual concentration in solution; q_{max} is the maximum specific uptake corresponding to the site saturation and b is the ratio of adsorption and desorption rates.

Freundlich:

$$q = K_F C_{eq}^{1/n}$$

where K_F and n are constants.

These models can be applied at a constant pH, and used for modeling of biosorption equilibrium in the presence of one metal.

Biosorption isothermal constants for Langmuir model and Freundlich model reported in the literature for precious metal biosorption are given in Table 5.

7. Mechanism of biosorption

The mechanism of metal biosorption is a complicated process. The status of biomass (living or non living), types of biomaterials, properties of metal solution chemistry, ambient/environmental conditions etc. influence the mechanism of metal biosorption. The mechanisms of biosorption are generally based on physico-chemical interactions between metal ions and the functional groups present on the cell surface, such as electrostatic interactions, ions exchange and metal ion chelation and complexation (Ozer et al., 2004). Biosorption of precious metals from solutions can be categorized as physical adsorption mechanism and chemical absorption mechanism or chemisorption. Physical sorption mechanisms generally involve electrostatic forces and ion exchange while chemisorption mechanisms include complexation, chelation and microprecipitation while (Kratochvil and Volesky, 1998; Kuyucak and Volesky, 1988). Some of the possible mechanisms of precious metal uptake processes discussed by different workers have been reported here.

Several spectroscopic methods of analysis have been used to study the mechanisms of gold and silver biosorption and reduction processes. Pethkar et al. (2001) reported the gold and silver adsorption capacities of the two strains of a fungus *Cladosporioum cladosporoides* (strain 1) and *Cladosporioum cladosporoides* (strain 2). X ray photoelectron spectroscopy (XPS) and Fourier transform infra red spectroscopy (FTIR) analysis indicated that protonated carbonyl and carboxyl groups played a key role in gold biosorption under acidic condition in strain 1 whereas in case of strain 2, involvement of C–O and C–N bonds were noted. The cell wall hexosamine content was found to be 150 times more in the strain 2 confirmed the role of hexosamines in precious metal binding.

The mechanism for Au (III) biosorption and reduction on alfalfa biomass was reported by Gardea-Torresdey et al. (2002). X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy studies suggested that gold(III) was initially reduced to gold(I) and then eventually reduced to gold (0). In addition, the X-ray spectroscopic studies indicated that binding might be occurred through a nitrogen or oxygen ligand. Arrascue et al. (2003) studied that in acidic solutions, chitosan was protonated and protonated amine groups were available for sorption of anionic gold species. Glutaraldehyde cross-linked chitosan could enhance the mechanism but the sorption capacity strongly decreased with increasing pH.

Spectroscopic studies were carried out by Romero-Gonz´alez et al. (2003) using dealginated seaweed waste on biosorption of gold (III). Colloidal gold was formed on the surface of dealginated seaweed by reduction of Au (III) to Au (0). Four different types of gold particles viz. hexagonal platelets, tetrahedral, rods and decahedral were clearly identified by ESEM spectroscopy. Presence of nearly 75% of colloidal gold was measured through X-ray absorption fine structure (EXAFS) spectroscopy. Reduction of gold from Au (III) to Au (I) and Au (0) was also confirmed by the measured bond distances characteristic of the metal.

XRD and FTIR spectroscopic analysis was used by Atia et al. (2005) to study the biosorption mechanism of Au^{3+} ions on waste biomass of *Saccharomyces cerevisae*. FTIR spectrophotometry demonstrated that active groups such as the hydroxyl group of saccharides and the carboxylate anion of amino-acid residues from the peptidoglycan layer on the cell wall were the sites for the Au (III) binding, and the free aldehyde group acted as the electron donor for reduction of Au (III) to Au (0). X-ray diffraction pattern gave peaks exactly corresponding to Au (0) thereby supporting bioreduction.

Table 5
Isothermal constants of Langmuir model and Freundlich model for precious metal biosorption (Cui and Zhang, 2008).

Precious metals	Biosorbents	pH	Langmuir parameters			Freundlich parameters			Reference
			qmax (mg/g)	b (L/mg)	R^2	n	K^c	R^2	
Au3+	*Saccharomyces cerevisiae*	5	5.55	0.00259	–	–	–	–	Savvaidis (1998)
Au3+	*Spirulina platensis*	4	5.55	0.0129	–	–	–	–	Savvaidis (1998)
Au3+	*Streptomyces erythraeus*	4	6.00	0.00472	–	–	–	–	Savvaidis (1998)
Au3+	PVA-immobilized fungal biomass	–	94.34	0.1125	0.9466	37.17	0.182	0.9158	Khoo and Ting (2001)
Au+	Eggshell membrane	3	226	0.2213	0.976	–	–	–	Ishikawa et al. (2002)
Au+	Eggshell membrane	3	132	0.0146	0.997	–	–	–	Ishikawa et al. (2002)
Pt4+	*Desulfovibrio Desulfuricans*	2	62.5	0.50	2.27	24.2	0.292	2.66	de Vargas et al. (2004)
Pt4+	*Desulfovibrio fructosivorans*	2	32.3	1.17	2.66	20.3	0.140	1.95	de Vargas et al. (2004)
Pt4+	Bayberry tannin Immobilized collagen fiber (BTICF) membrane	3	45.8	0.21	0.991	–	–	–	Ma et al. (2006)
Pd2+	*Desulfovibrio Desulfuricans*	2	128.2	0.9	6.20	65.5	0.224	11.23	de Vargas et al. (2004)
Pd2+	*Desulfovibrio Fructosivorans*	2	126.5	0.17	8.44	22.8	0.321	13.51	de Vargas et al. (2004)
Pd2+	*Desulfovibrio vulgaris*	–	108.6	0.56	7.16	38.0	0.275	6.06	de Vargas et al. (2004)

Binding mechanism of gold with tannin gel particles was highlighted by Ogata and Nakano (2005).The results suggested that a redox reaction between tannin gel and $AuCl^{4-}$ was responsible for reduction of Au (III) ions to Au (0) ions and oxidation of hydroxyl groups of the tannin gel to carbonyl groups.

Gold recovery from cyanide solution by bacterial consortium was studied by Aitimbetov et al. (2005).The main mechanism underlying this process was the microbial consortium which could degrade the metal cyanide formed by gold and release gold ions.

$$[Au(CN)_2]^- + aH_2O + b\,O_2 + \text{enzyme} \dashrightarrow Au^+ + cNH_4^+ + dCO_2 + \text{cellmass}$$

The gold ions aggressively seek an electron from the close surroundings and become stabilized in the metallic form as follows: $Au^+ + e^- - Au^0$.

Role of anaerobic bacterial species *Shewanella algae* in biosorption of $AuCl^{4-}$ ions and intracellular precipitation was studied by Konishi et al. (2006). TEM & EDX analysis reported the presence of gold nanoparticles.

Biosorption properties of chemically modified chitosan was studied by Donia et al. (2007). Uptake of 3.6 mmol/g gold was reported through a series of experiments conducted in batch and column mode. Since the uptake process took place in a strong acid medium, the dominant mechanism of interaction was probably due to salt formation ($R-CH=NH^+$ $AuCl^{4-}$). Persimmon peel gel is also a gold recovering agent as studied by Parajuli et al. (2007). An XRD analysis and digital micrograph analysis showed the presence of gold particles on the gel.

Gold (III) biosorption and bioreduction with brown algae *Fucus vesiculosus* have been reported by Mata et al. (2009). Hydroxyl groups present in the algal polysaccharides were found to be involved in the gold bioreduction. Metallic gold was detected as microprecipitates on the biomass surface and in colloidal form as nanoparticles in the solution.

Silver recovery from $AgNO_3$ and Ag $(S_2O_3)_2^{3-}$ using two strains of microalgae *Chlorella vulgaris* was studied by Cordery et al. (1994). A comparison was drawn between the two strains and it was reported that the second strain gave a higher uptake percentage owing to a higher number of positive surface charges compared to strain 1. Due to more surface positivity of strain 2, it could biosorb silver thiosulfate unlike strain 1. Mechanism of silver biosorption was reported by Tsezos et al. (1997) on three bacterial strains namely *Arthrobaccer* sp, *Alkaligenes eutrophus* and *Pseudomonas mendocina* .The inference was drawn as a result of TEM microscopic analysis and EDAX analysis. A further in depth study was done by Kasthuri et al. (2009) who used FTIR and TEM analysis to prove that silver precipitated as nanoparticles as a result of interaction of Ag^+ with carbonyl groups. A similar sort of interaction of Ag with carboxylate group on *Lactobacillus* sp. was investigated by Lin et al. (2005b). FTIR, XPS and XRD analysis proved that reduction of Ag (1) to Ag (0) accompanied the process of biosorption. Donia et al. (2007) reported that modification of chitosan by a reaction between chitosan and polymeric Schiff's base of thiourea/glutaraldehyde in the presence of magnetite enhanced the process of silver uptake by modified chitosan.

In case of platinum and palladium biosorption, electrostatic interaction plays a major role. Biosorption of platinum by chitosan cross-linked with glutaraldehyde was studied by Guibal et al. (1999a). At an acidic pH, an uptake of 300 mg/g was reported. Presence of chloride and nitrate ions decreased the platinum uptake due to competition for positive sites thereby proving the phenomena of electrostatic binding mechanism. Similar work was done by Fujiwara et al. (2007) on chitosan modified by cross linking with l-lysine .They reported that N atoms of amino group and O atoms of carboxyl group was mostly responsible for the biosorption. This was confirmed by FTIR analysis as C–N and C–O bond stretching. New chelating and reactive functional groups can readily be-grafted onto the chitosan backbone on amine and/or hydroxyl groups (Guibal et al., 2002).) A variety of chitin derivatives namely glutaraldehyde cross-linking (GA), poly (ethyleneimine) grafted (PEI) and thiourea grafted (TDC) has been studied by Chassary et al. (2005). The sorption mechanism of both Pt (II) and Pd (II) involved the formation of chloro-anion complexes which got sorbed on the positively charged amino groups. This was simultaneously accompanied by chelation of the metal ion on sulphur containing groups on TDC. Parsons et al. (2003) reported that esterification process converting the alfalfa biomass carboxylic groups to methyl esters, allowing Pt (II) to get closer to the esterified biomass and to bind in greater amounts at higher pH. Similar work was done by Arrascue et al. (2003) who grafted sulfur moites onto chitosan and found a mechanism shift.

Although the electrostatic mechanism seems to be generally accepted, a different situation was presented by some other workers where chemical mechanisms and redox reactions were implicated using tannin to sorb platinum and palladium. Wang et al. (2005) reported the remarkable selectivity of collagen fiber immobilized bayberry tannins for biosorption of Pt (II) and Pd (II). Kim and Nakano (2005) applied tannin gel particles to palladium recovery and the mechanism was very similar to gold reduction mechanism where a redox reaction resulted in the reduction of Pd (II) to Pd (0). XRD analysis for the tannin gel particles that adsorbed palladium were sampled to find out the chemical form of the adsorbed palladium. Different peaks corresponding to crystalline metallic palladium confirmed the crystallization of reduced Pd (0) on tannin gel network Reduction of palladium (II) to palladium (0) was confirmed by the presence of metallic palladium on the tannin gel particles, while the hydroxyl groups of tannin gel were oxidized by redox-reaction during the adsorption.

Again, it seemed that the mechanism responsible for platinum and palladium binding depends largely on the type or form of the sorbent used (Mack et al.,2007). Platinum biosorption on the PEI-modified biomass was explained by the electrostatic attraction of anionic Pt-chloride complexes to protonated amine groups on the biomass surface (Won et al., 2009).

8. Desorption and recovery

Desorption is very much necessary when the biomass preparation/generation is costly. It is possible to decrease the process cost and also the dependency of the process on a continuous supply of the biosorbent through desorption. A successful desorption process requires the proper selection of elutants, which strongly depends on the type of biosorbent and the mechanism of biosorption. Elutant must be i) non damaging to the biomass ii) less costly, iii) eco-friendly and iv) effective. Some workers have conducted exhaustive experiments to identify appropriate elutants for the recovery of precious metals.

Ichikawa et al. (2002) reported that desorption of gold was very effective using NaOH and NaCN. The NaOH solution (0.1 mol/l) was capable of desorbing more than 95% of the gold (I) sorbed to egg shell membrane (ESM) and NaCN (0.1 mol/l) was capable of desorbing more than 92% of the gold (III). ESM column was used and 98% recovery of gold was reported.

The elution of gold sequestered on brown marine alga, *Saragasum natans*, was done by a solution of 0.1 mol/l thiourea with 0.02 mol/l ferric ammonium sulfate; However, the optimum time for desorption was determined to be as long as 17 h (Kuyucak and Volesky, 1989).

Batch desorption experiments were tried by using 0.1 M HCl, 1.0 M HCl, 0.1 M EDTA and 0.1 M thiourea–1.0 M HCl, respectively for the recovery of platinum and palladium (Ma et al.,2006). It was found that acidic thiourea solution exhibited effectiveness for the regeneration of the bayberry tannin immobilized collagen fiber (BTICF) membrane material. In the case of Pt (IV), desorption extent was 90.7%, but it was only 58.4% for Pd (II).

Silver recovery by internal electrolysis with cassava-derived cyanide solution was recommended for both solid and effluent wastes from industries (Ajiwe and Anyadiegwu , 2000).

Experiments on desorption of gold and silver ions were conducted by Donia et al. (2007). Sorption/ desorption cycles up to four runs were carried out for Au (III) and Ag (I) on chemically modified chitosan resin. The elution of the metal ions was performed well using 100 mL of 0.5 M thiourea acidified with drops of 0.2 M H_2SO_4. This indicated that the resin had good durability as well as good efficiency for repeated use.

9. Continuous biosorption

To evaluate the technical feasibility of biosorption process for real applications, continuous biosorption studies gains utmost importance. Packed bed columns have been established as an effective, economical and most convenient for biosorption processes among the different column configurations (Saeed and Iqbal, 2003; Chu, 2004). Biosorption through Packed bed column has a number of process engineering merits, including a high operational yield and the relative ease of scaling up procedures (Aksu, 2005). Other column reactors, such as fluidized and continuous stirred tank reactors are very rarely used for the purpose of biosorption (Prakasham et al., 1999; Solisio et al., 2000). Continuous stirred tank reactors are useful when biosorbent is in the form of powder (Cossich et al., 2004); however, they suffer from high capital and operating cost (Volesky, 1987). Fluidized bed systems, which operate continuously require high flow rates to keep the biosorbent particles in suspension (Muraleedharan et al., 1991).

The continuous adsorption experiments containing 58.3 mg/L Pt (IV) and 48.8 mg/L Pd(II) mixture solutions were carried out by Ma et al. (2006) using three layers of bayberry tannin immobilized collagen fiber (BTICF) membrane (total area 191 cm^2). No Pt (IV) and Pd (II) ions were detected until 700 and 1000 ml effluents were collected, respectively. Furthermore, competitive adsorption studies revealed that the BTICF membrane exhibited ability of selective adsorption to Pt (IV) and Pd (II) in the mixture solutions of metal ions which would be practically useful for separation of precious metals from the mixture solution of metal ions.

A columnar electrobioreactor having 2 L capacity was constructed by Creamer et al. (2006) to study the recovery of gold and palladium using *Desulfovibrio desulfuricans*.

Donia et al. (2007) conducted continuous biosorption studies using chemically modified chitosan resin with magnetic properties for the recovery of gold and silver. Breakthrough time, critical bed height and durability of resin were estimated from column studies. The breakthrough curves for recovery of Au (III) and Ag (I) showed no appreciative changes during successive cycles which confirmed the potentiality of resin for reuse.

10. Application of biosorption using real industrial effluents /wastes

Biosorption is a proven technique for recovery of precious metals from aqueous solutions. However, its performance under real wastewater conditions is of concern. There have been few investigations examining the compatibility of the biosorbents used for the recovery of precious metals from real industrial effluents.

In order to test the applicability of the fungal biosorbent *Cladosporium cladosporioides* biomass beads on gold sorption, studies were carried out by Pethkar and Paknikar (1998) using an electroplating effluent (containing 46 mg l^{-1} gold). In batch experiments, the uptake of gold by the biosorbent beads was found to be 55%. The observed gold loading capacity was 36 mg g^{-1}.

Recovery of silver from industrial waste was reported by Ajiwe and Anyadiegwu (2000). Silver was recovered from X-ray films by dissolving the silver compounds with concentrated nitric acid. Internal electrolysis and external electrolysis methods were carried out using a steel electrode as cathode and a copper electrode as anode. Addition of cassava-derived cyanide solution proved to give a better recovery in both methods. The results showed that external electrolysis gave a better recovery. However, on the basis of energy consumption, silver recovery by internal electrolysis with cassava-derived cyanide solution was recommended for both solid and effluent wastes from industries.

Ishikawa et al. (2006) reported the selective recovery of gold from electroplating wastewater containing different metal ions using egg shell membrane column. The recovery order was Au 98%>Ag 97%>Co 94%>Cu17%>Pb 15%>Ni 4%>Zn 3%.

The activated hard shell of Iranian apricot stones was used by Soleimani and Kaghazchi (2008) for recovery of gold from electroplating wastewater. The results suggested that activated hard shell of Iranian apricot stones could be used as an effective substitute as an adsorbent for other imported commercial activated carbons due to its physical and chemical properties and adsorptive properties with respect to adsorption of gold ions.

Won et al. (2010) reported the recovery of platinum from wastewater collected from an industrial laboratory for inductively coupled plasma (ICP) analysis. Polyethylenimine (PEI)-modified biomass, a high performance biosorbent, was prepared by attaching PEI onto the surface of inactive *Escherichia coli* biomass, which was formed in the L-phenylalanine fermentation industry. *E. coli* biomass was chosen because a large quantity of waste *E. coli* is generated as a result of large-scale fermentation. The maximum platinum uptake of PEI-modified biomass was enhanced up to 108.8 mg/g compared to 21.4 mg/g of the raw biomass.

11. Conclusions

Biosorption of precious metals from solutions has received great deal of attention in the recent years. Compared with the conventional methods, a biosorption-based process offers a number of advantages including low operating costs, minimization of the volume of chemical and/or biological sludge to be handled and high efficiency in detoxifying effluents.

Research over the past decade has provided a better understanding of precious metal recovery through biosorption using certain potential biosorbents. The groups of cheap biomaterials constitute the basis for a new cost effective technology that can find its application in the recovery of precious metal containing industrial effluents.

Despite continuing dramatic increases in published research work on biosorption, there has been little exploitation in an industrial context. A number of biosorbents have been reported for recovery of precious metals, but there have only been few instances where biosorption processes have managed to reach commercialization. Two commercial biosorbents presently available include AlgaSORB™ (biomass of *Chlorella vulgaris*) and AMT-Bioclaim™ (biomass of *Bacillus subtilis*) (Kuyucak, 1997). Both the biosorbents can efficiently sequester metal ions from dilute solutions (10 to 100 mg L^{-1}). AMT-Bioclaim™ is capable of accumulating gold, cadmium and zinc from cyanide solutions and is therefore suitable to metal finishing operations. To attract more usage of biosorption technology, strategies have to be developed where further processing of biosorbent can be done to regenerate the biomass and then convert the recovered metal into usable form which can be sold in the market.

The mechanisms of biosorption of precious metals need to be further elucidated. Molecular biotechnology, a powerful tool to elucidate the mechanism at molecular level should be considered more in the future to construct an engineered organism with higher biosorption capacity and specificity for target precious metal ions. Immobilization of biomaterials is another key aspect for the purpose of biosorption application. It is important for decreasing the cost of immobilization and consequently distribution, regeneration and

reuse of biosorbents. Although continuous process of immobilized cells has been realized at lab scale, there is still a long way to go for biosorption commercialization.

The potential application for biosorption appears to be enormous. The easy operation and cost advantage of biosorption technology would guarantee a strong penetration of large market for precious metal recovery in future.

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

SANITARY DISTRICT OF DECATUR,	)	
	)	
Petitioner,	)	
	)	PCB 14-111
v.	)	(Variance - Water)
	)	
ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,	)	
	)	
Respondent.	)	

CERTIFICATE OF SERVICE

I, the undersigned, an attorney, state that I have served electronically the attached Recommendation of the Illinois Environmental Protection Agency upon:

John Therriault, Assistant Clerk
Illinois Pollution Control Board
James R. Thompson Center
100 West Randolph, Suite 11-500
Chicago, Illinois 60601

via electronic mail on April 7, 2014; and depositing said documents in the United States Mail, postage prepaid, in Springfield Illinois, on April 7, 2014 to each persons on the attached service list.

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

DATED: April 7, 2014

By: Sara Terranova

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