BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
PROPOSED AMENDMENTS TO GROUNDWATER QUALITY (35 ILL. ADM. CODE 620)	)	**R 2022-018 (Rulemaking - Public Water Supply)**

NOTICE OF FILING

To: ALL PARTIES ON THE ATTACHED SERVICE LIST

PLEASE TAKE NOTICE that I have today electronically filed with the Office of the Clerk of the Illinois Pollution Control Board the attached **Dynegy's Comments to the Proposed Second Notice**, copies of which are hereby served upon you.

/s/ Bina Joshi
Bina Joshi

Dated: December 6, 2024

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Dynegy's Comments to the Proposed Second Notice

Dynegy Midwest Generation, LLC; Electric Energy Inc.; Illinois Power Generating Company; Illinois Power Resources Generating, LLC; and Kincaid Generation, LLC (collectively, "Dynegy"), collectively submit these Comments to the Illinois Pollution Control Board's (the "Board's") Proposed Second Notice Opinion and Order in R22-18 ("Proposed Second Notice").

Comment 1 – The Second Notice Rulemaking Should Incorporate Dynegy's Proposed Amendments to IEPA's Language Regarding the Interplay between Part 845 and Part 620.

I. Revisions to 620.240 and 620.440

Illinois Environmental Protection Agency's ("IEPA's") First Notice Comment proposed revisions to 620.240 and 620.440 that would classify groundwater impacted by CCR surface impoundments regulated under Part 845 as Class IV groundwater. (P.C. 63). These revisions are intended to clarify the interplay between Part 845 and Part 620. In its Proposed Second Notice, the Board explained:

> The Board agrees with IEPA that the proposed inclusion of groundwater impacted by CCR surface impoundments regulated under Part 845 Class IV groundwaters and the corresponding addition of Class IV GWQS applicable to Part 845 surface impoundments alleviates potential confusion between the applicability of Part 845 GWPS and Part 620 GWQS. Therefore, the Board accepts IEPA's suggested amendments to Sections 620.240 and 620.440 with non-substantive changes. However, the Board asks IEPA to comment on why the Board should not include the[sic] Dynegy's revisions to IEPA's language.

Proposed Second Notice at 22. In its First Notice Comment, Dynegy was generally supportive of IEPA's proposed amendments to 620.240 and 620.440, but pointed out that revisions to IEPA's proposed language are necessary to provide clarity that both *onsite* and *offsite* groundwater regulated under Part 845 would be classified as Class IV. (P.C. 66). As IEPA explained in its First Notice comment

> groundwater regulated under Part 845 falls within a specific area at electric utilities and independent power producers, is associated with one or more CCR surface impoundments, is being monitored up gradient, cross-gradient and down gradient of the CCR surface impoundments by an Agency approved groundwater monitoring system and includes all of the of groundwater impacted by releases from

> a CCR surface impoundment. This may include groundwater both onsite and offsite at an electric utility or independent power producer.

(P.C. 63 at 11) (emphasis added). Part 845 regulates groundwater contaminated by CCR surface impoundments both onsite (at the property of an electric utility or independent power producer) and that travels offsite to other properties. *See e.g.* 35 Ill. Adm. Code §§ 845.650(d), 845.700(g)(5).

To avoid ambiguity, Dynegy recommended the following revisions to IEPA's proposed Sections 620.240(h) and 620.440(d):

> 620.240
>
> Except as provided in Section 620.250, Other Groundwater is:
>
> h) Groundwater regulated under 35 Ill. Adm. Code 845 ~~at both active and inactive electric utilities and independent power producers~~.
>
> 620.440
>
> d) For groundwater ~~at both active and inactive electric utilities and independent power producers~~ regulated under Part 845, the groundwater protection standard (GWPS) under Section 845.600 must not be exceeded for any constituent with a GWPS under Section 845.600. For any constituent that does not have a GWPS under Section 845.600, the groundwater quality standards (GWQS) of Sections 620.410, 620.420, 620.430 or 620.440(b) and (c) apply.

The struck language is unnecessary and could create ambiguity if left in as is. All groundwater regulated by Part 845 should be clearly captured under the language in 620.240(h) and 620.440(d), including (1) groundwater regulated under Part 845 located at active and inactive electric utilities and independent power producers and (2) groundwater regulated under Part 845 located *offsite* from those active and inactive electric utilities and independent power producers. If not, these sections could be incorrectly interpreted as resulting in the simultaneous application Part 845 and Part 620 when contamination regulated and being cleaned up under Part 845 migrates offsite – the opposite of the Board and IEPA's intention. As an alternative, these provisions could also be revised as follows, to provide the same clarity regarding application to groundwater regulated under Part 845 that has traveled offsite:

> 620.240
>
> Except as provided in Section 620.250, Other Groundwater is:
>
> h) Groundwater regulated under 35 Ill. Adm. Code 845 at or from both active and inactive electric utilities and independent power producers.
>
> 620.440

d) For groundwater at or from both active and inactive electric utilities and independent power producers and regulated under Part 845, the groundwater protection standard (GWPS) under Section 845.600 must not be exceeded for any constituent with a GWPS under Section 845.600. For any constituent that does not have a GWPS under Section 845.600, the groundwater quality standards (GWQS) of Sections 620.410, 620.420, 620.430 or 620.440(b) and (c) apply.

II. Possible Reconsideration of 620.250(i)

IEPA also proposed revision to 620.250(i) to address that "no new GMZ under Part 620.250 should be issued for the same constituents required under Part 845" and to avoid simultaneous remediation of constituents under Part 620 and Part 845. (P.C. 63 at 20). The Board did not adopt this language into its Proposed Second Notice, and Dynegy agrees the revision is unnecessary because this issue is already addressed under language in Part 845 and the clarifications to Part 620 classifying groundwater/constituents regulated under Part 845 as Class IV. *See* 35 Ill. Adm. Code §845.600 (c) and discussion above.

However, if the Board reconsiders this language as part of comments to its Proposed Second Notice, IEPA's proposed language for 620.250(i) must be amended, as follows, to avoid confusion and align with IEPA's intent that a groundwater management zone ("GMZ") not be available for constituents that are *in groundwater regulated under Part 845*:

620.250:

i) Regardless of subsections (a) through (f), any corrective action conducted under 35 Ill. Adm. Code 845 must follow the corrective action process of Sections 845.650, 845.660, 845.670, and 845.680. A GMZ will not be approved for any constituent in groundwater regulated under ~~with a~~ Part 845~~ Groundwater Protection Standard (GWPS)~~. A site owner or operator may apply for a GMZ for any constituent with no Part 845 Groundwater Protection Standard (GWPS) subject to the requirements of subparts (a) through (f).

Without these revisions, the second sentence of IEPA's proposed language could lead to the unintended conclusion that a GMZ is never available for a constituent that has both a Part 845 Groundwater Protection Standard and a Part 620 Groundwater Quality Standard, regardless of whether that constituent is located in groundwater regulated under Part 845. For example, boron has a Part 845 Groundwater Protection Standard and a Part 620 Groundwater Quality Standard. Without revision, the second sentence of IEPA's proposed language could be interpreted as excluding the owner or operator of a manufacturing site with boron exceedances, caused by manufacturing activities, from making use of a GMZ. This conclusion is clearly not IEPA's intent.

Thus, again, Dynegy agrees that the language in this provision is unnecessary. But, if the Board for any reason reconsiders this language, it should do so with the revisions above to avoid confusion, clarify the provision's intent and provide necessary clarity.

Comment 2 – The Board Should Revise the Class I and II Molybdenum Standard in its Proposed Second Notice

Dynegy agrees with the comments provided by the International Molybdenum Association on November 15, 2024. (P.C. 72). The Board should revise the molybdenum standards included in its Proposed Second Notice for greater consistency with currently accepted scientific studies and data. The Board appropriately used 2020 ATSDR MRL ("ATSDR MRL") to set a Class I molybdenum standard. However, the human threshold toxicant advisory concentration ("HTTAC") calculation provided by IEPA, using the ATSDR MRL to reach a standard of 0.023 mg/L, uses an inappropriate uncertainty factor and relative source contribution. As explained below (and for the reasons explained in P.C. 72), a Class I molybdenum standard of 0.231 mg/L or 0.923 mg/L is far more appropriate and supportable.

I. No uncertainty factor should apply to the HTTAC calculation

IEPA's calculation applied an uncertainty factor of 10 to extrapolate from a subchronic RfD/MRL to a chronic Rfd/MRL. (P.C. 71). However, as the United States Environmental Protection Agency ("USEPA") Region 8 and European Chemicals Agency ("ECHA") have explained, no uncertainty factor should be applied when calculating a risk-based molybdenum standard due to the availability of chronic data demonstrating no uncertainty factor is required.

USEPA Region 8 has concluded no uncertainty factor is necessary when setting a risk-based molybdenum water quality standard. USEPA weighed in on this issue when Colorado derived a water quality standard for molybdenum based on the ASTDR MRL. Colorado did not apply an additional uncertainty factor when developing its risk-based molybdenum standard. (*See* P.C. 62[1]). USEPA Region 8 accepted Colorado's standard and commented that no uncertainty factor was needed because of the availability of other chronic data. Specifically, the National Toxicology Program's 1997 chronic study found there were no further adverse effects from molybdenum in the kidney, which is the most sensitive endpoint.[2] USEPA Region 8 explained:

> The evidence available from molybdenum studies for kidney health effects, the most sensitive endpoint reported following repeated molybdenum exposure (ATSDR, 2020), supports the conclusion that application of an uncertainty factor to account for subchronic to chronic exposures is unnecessary for the intermediate duration oral MRL.

(P.C. 62). Similarly, ECHA declined to apply an additional uncertainty factor to the ATSDR MRL when deriving a risk-based molybdenum standard. Like USEPA Region 8, they declined due the

[1] Ref: 8WD-CWQ SENT VIA EMAIL to Mike Weber, Chair Colorado Water Quality Control Commission f cdphe.wqcc@state.co.us From Andrew Todd Water Quality Division Subject: Proposed Changes to Molybdenum WQS and Enclosure EPA Region 8 Comments on Proposed Changes to Molybdenum WQS, attached to P.C. 62.

[2] NTP. 1997. Toxicology and carcinogenesis studies of molybdenum trioxide in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: National Toxicology Program. TR263. available at https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr462.pdf, attached as Exhibit 1 (without appendices).

level of evidence from the National Toxicology Program 1997 inhalation study demonstrating no further adverse effects are expected following chronic exposures. Thus, USEPA, ECHA and Colorado have engaged in detailed consideration and technical review to determine whether an uncertainty factor should be applied when deriving a health-based groundwater standard for molybdenum and they have each concluded that the application of any uncertainty factor is inappropriate.

If the HTTAC for molybdenum is recalculated using the same assumptions as IEPA in P.C.71 (i.e. MRL of 0.06 mg/kg-day, RSC of 0.2, 0.78 liters of water/day for a child (0-6 years of age), equal to 15) but without the unnecessary application of an uncertainty factor of 10, the result is a water quality concentration for molybdenum of 0.231 mg/L.

II. The Relative Source Contribution Factor Applied to the HTTAC Calculation for Molybdenum Should be 0.8

IEPA applied a Relative Source Contribution ("RSC") of 0.2 when performing the HTTAC calculation for molybdenum. However, evidence from USEPA Region 8 in support of Colorado's molybdenum standard demonstrates that an RSC of 0.8 is more appropriate. IEPA dismisses the use of the 0.8 RSC utilized by Colorado and supported by USEPA by suggesting the 0.8 RSC should not apply because (1) IEPA's proposed standard is based on child exposures not adult exposures and (2) ATSDR's molybdenum toxicological profile makes a statement that exposure to molybdenum in the general population is almost entirely through food. (P.C. 71 at 3). However, these statements ignore the reliable, and more detailed data provided in USEPA Region 8's discussion of the Colorado drinking water standard. This data demonstrates that the intake of molybdenum from food sources is significantly less than 20% for individuals of all ages (including children). [3]

> As shown in Table-1[4], while TVS values calculated for different life stages change based upon the ratio of body weight to drinking water intake rate, the relative contribution of molybdenum estimated to come from the diet *remains below 20% across all lifestages*, and below 10% for all but potentially bottle-fed infants.

[3] *See* Ref: 8WD-CWQ SENT VIA EMAIL to Mike Weber, Chair Colorado Water Quality Control Commission f cdphe.wqcc@state.co.us From Andrew Todd Water Quality Division Subject: Proposed Changes to Molybdenum WQS and Enclosure EPA Region 8 Comments on Proposed Changes to Molybdenum WQS, attached to P.C. 62.

[4] Table 1, attached to P.C. 62, references include: Abramovich M, ea. (2011). Molybdenum content of Canadian and US infant formulas. Biol Trace Elem Res. Nov;143(2):844-53. doi: 10.1007/s12011-010-8950-4. Epub 2011 Jan 29. PMID: 21279467.
Agency for Toxic Substances and Disease Registry (ATSDR) (2020). Toxicological Profile for Molybdenum, TP212. May 2020.
NIH (2024). Molybdenum, Fact Sheet for Health Professionals. Accessed March, 2024. https://ods.od.nih.gov/factsheets/Molybdenum-HealthProfessional/#h2USEPA (2011). Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Office of Research and Development. September, 2011. EPA/600/R-09/052F.
USEPA (2019). Update for Chapter 3 of the Exposure Factors Handbook: Ingestion of Water and Other Select Liquids. National Center for Environmental Assessment, Office of Research and Development. February, 2019. EPA/600/R-18/259F.

Table 1 Evaluation of Lifestage-Specific Exposures and Molybdenum (Mo) Intake

Lifestage	Ages	Body weight (average, kg)[a]	Water intake (90%-ile, L/day)[b]	BW / WI ratio[d]	Lifestage-specific-TVS[e] (µg/L)	Total allowable daily intake (ADI) (µg)[f]	Amount of Mo intake from diet (µg)[g]	Percent of Mo intake from diet in ADI
Adult	≥ 21 years	80	2.4	33.3	1,600	4,800	180 – 360	3.8 – 7.5%
Child	6 – 11 years	31.8	0.953	33.4	1,603	1,900	102	5.4%
Child	3 – 6 years	18.6	0.683	27.2	1,306	1,100	66	6%
Infant (breast-fed)	3 – 6 months	7.4	1.037[c]	7.14	343	440	5	1.1%
Infant (bottle-fed)	3 – 6 months	7.4	1.3[c]	5.69	273	440	49	11%

[a] From USEPA (2011); kilograms, kg
[b] From USEPA (2019); liters per day, L/day; 90th percentile of the population, 90%-ile.
[c] Estimates noted as less reliable
[d] Ratio of body weight (BW) / water intake (WI) from TVS equation 1, as a measure of relative drinking water exposure
[e] As shown in Equation 1-1 in WQCC Policy 96-2; with RfD = 0.06 mg/kg-day, RSC = 0.8, additional UF=1x, and all other parameters as noted in the table.
[f] For molybdenum, based upon RfD of 0.06 mg/kg-day and body weight indicated in the table; allowable daily intake (ADI).
[g] Little lifestage specific data available: for children 6-11 years, assumed dietary intake of 3x the recommended daily intake of 34 µg/day for ages 9 – 13 years, and for children 3-6 years assumed dietary intake of 3x the recommended daily intake of 22 µg/day for ages 4 – 8 years (NIH, 2024); for breast-fed infants 3 – 6 months, assumed intake from human breast milk average of 5 µg/L; for bottle-fed infants 3 – 6 months assumed intake from infant formula average of 38 µg/L (Abramovich M et al., 2011)

This detailed evidence from USEPA Region 8 demonstrates that an RSC of 0.8 is conservatively protective.

If the HTTAC for molybdenum is recalculated using the same assumptions as IEPA in P.C. 71 but adjusted to remove the uncertainty factor and to assign an RSC of 0.8 (i.e. MRL of 0.06 mg/kg-day, an RSC of 0.8, 0.78 liters of water a day for a child (0-6 years of age), equal to 15 kg, with no uncertainty factor) the resulting acceptable water quality concentration for molybdenum is 0.923 mg/L.

III. The ATSDR MRL Already Accounts for Copper Intake / No Copper Intake-Related Adjustments are Needed

Finally, the Board does not apply any modifying factor to account for copper intake in setting the molybdenum standard in its Proposed Second Notice, nor should it.

The ATSDR MRL already includes consideration of individuals with lower copper intake. The ATSDR MRL conservatively applies a modifying factor of 3 to address concerns that reproductive and/or developmental effects may be a more sensitive endpoint than kidney effects in populations with marginal copper intakes. Further, evidence from the National Institutes of Health Office of Dietary Supplements has found the actual intake of copper in both children and adults is higher than the recommended intake, demonstrating individuals are achieving sufficient copper intake in

the United States.[5] Given the data of sufficient copper intake and the fact that the ATSDR already considered the possibility of lower copper intake by applying a modifying factor of three, the application of any additional modifying factor to the ATSDR MRL for copper is unnecessary.

Respectfully submitted,

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[5] NIH, Copper Fact Sheet for Health Professionals, available at https://ods.od.nih.gov/factsheets/Copper-HealthProfessional/, attached as Exhibit 2.

CERTIFICATE OF SERVICE

I, the undersigned, certify that on this 6th day of December, 2024, I have served electronically the attached **Dynegy's Proposed Second Notice Comment**, upon the individuals on the attached service list. I further certify that my email address is bina.joshi@afslaw.com; the number of pages in the email transmission is 95; and the email transmission took place today before 5:00 p.m.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

MOLYBDENUM TRIOXIDE

(CAS NO. 1313-27-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

April 1997

NTP TR 462

NIH Publication No. 97-3378

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Listings of all published NTP reports and ongoing studies are also available from NTP Central Data Management. The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

NTP TECHNICAL REPORT

ON THE

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MOLYBDENUM TRIOXIDE

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IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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Research Triangle Park, NC 27709

April 1997

NTP TR 462

NIH Publication No. 97-3378

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CONTENTS

ABSTRACT

MoO_3

MOLYBDENUM TRIOXIDE

CAS No. 1313-27-5

Chemical Formula: MoO_3 Molecular Weight: 143.95

Synonyms: Molybdena; molybdenum anhydride; molybdenum (VI) oxide; molybdenum peroxide; molybdic acid anhydride; molybdic anhydride; molybdic oxide; molybdic trioxide; natural molybdite

Molybdenum is an essential element for the function of nitrogenase in plants and as a cofactor for enzymes including xanthine oxidoreductase, aldehyde oxidase, and sulfide oxidase in animals. Molybdenum trioxide is used primarily as an additive to steel and corrosion-resistant alloys. It is also used as a chemical intermediate for molybdenum products; an industrial catalyst; a pigment; a crop nutrient; components of glass, ceramics, and enamels; a flame retardant for polyester and polyvinyl chloride resins; and a reagent in chemical analyses. Molybdenum trioxide was nominated by the NCI for toxicity and carcinogenicity studies as a representative inorganic molybdenum compound. The production of molybdenum trioxide is the largest of all the molybdenum compounds examined.

Male and female F344/N rats and B6C3F$_1$ mice were exposed to molybdenum trioxide (approximately 99% pure) by inhalation for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

14-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 3, 10, 30, 100, or 300 mg molybdenum trioxide/m^3. Rats were exposed for 6 hours per day, 5 days per week, for a total of 10 exposure days during a 14-day period. All rats survived to the end of the study. The final mean body weights of male rats exposed to 100 mg/m^3 and male and female rats exposed to 300 mg/m^3 were significantly lower than those of the control groups. Male rats exposed to 300 mg/m^3 lost weight during the study. There were no clinical findings related to exposure to molybdenum trioxide. No chemical-related lesions were observed.

14-DAY STUDY IN MICE

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 3, 10, 30, 100, or 300 mg molybdenum trioxide/m^3. Mice were exposed 6 hours per day, 5 days per week, for a total of 10 exposure days during a 14-day period. All mice survived to the end of the study. Final mean body weights of male and female mice exposed to 300 mg/m^3 were significantly lower than those of the control groups. Male mice exposed to 300 mg/m^3 lost weight during the study. There were no clinical findings related to exposure to molybdenum trioxide. No chemical-related lesions were observed.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were exposed to molybdenum trioxide by inhalation at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6.5 hours per day, 5 days per week, for 13 weeks. All rats survived to the end of the study. The final mean body weights of exposed rats were similar to those of the control groups. No clinical findings related to molybdenum trioxide exposure were observed. There were no significant chemical-related differences in absolute or relative organ weights, hematology or clinical chemistry parameters, sperm counts or motility, or liver copper concentrations between control and exposed rats. No chemical-related lesions were observed.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to molybdenum trioxide by inhalation at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6.5 hours per day, 5 days per week, for 13 weeks. All mice survived to the end of the study. The final mean body weights of exposed mice were similar to those of the control groups. There were no chemical-related clinical findings. There were no significant differences in absolute or relative organ weights or sperm counts or motility between control and exposed mice. There were significant increases in liver copper concentrations in female mice exposed to 30 mg/m^3 and in male and female mice exposed to 100 mg/m^3 compared to those of the control groups. No chemical-related lesions were observed.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female F344/N rats were exposed to molybdenum trioxide by inhalation at concentrations of 0, 10, 30, or 100 mg/m^3. Rats were exposed for 6 hours per day, 5 days per week, for 106 weeks.

Survival, Body Weights, and Special Studies

Survival rates of exposed male and female rats were similar to those of the control groups. Mean body weights of exposed groups of male and female rats were similar to those of the control groups throughout the study. There was a significant exposure-dependent increase in blood molybdenum concentration in exposed rats. Blood concentrations of molybdenum in exposed male rats were greater than those in exposed female rats. There were no toxicologically significant differences in bone density or curvature between control and exposed rats.

Pathology Findings

The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were increased in male rats with a marginally significant positive trend. No increase in the incidences of lung neoplasms occurred in female rats. Incidences of chronic alveolar inflammation in male and female rats exposed to 30 or 100 mg/m^3 were significantly greater than those in the control groups. No nasal or laryngeal neoplasms were attributed to exposure to molybdenum trioxide. Incidences of hyaline degeneration in the nasal respiratory epithelium in 30 and 100 mg/m^3 males and in all exposed groups of females were significantly greater than those in the control groups. The incidences of hyaline degeneration in the nasal olfactory epithelium of all exposed groups of females were significantly greater than that in the control group. In the larynx, incidences of squamous metaplasia of the epithelium lining the base of the epiglottis in all exposed groups of male and female rats were significantly greater than those in the control groups and increased with increasing exposure concentration.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to molybdenum trioxide by inhalation at concentrations of 0, 10, 30, or 100 mg/m^3. Mice were exposed for 6 hours per day, 5 days per week, for 105 weeks.

Survival, Body Weights, and Special Studies

The survival rate of male mice exposed to 30 mg/m^3 was marginally lower than that of the control group; survival rates of 10 and 100 mg/m^3 males and of all exposed groups of females were similar to those of the control groups. Mean body weights of exposed male

mice were generally similar to those of the control group throughout the study. Mean body weights of exposed female mice were generally greater than those of the control group from week 11 until the end of the study. There was a significant exposure-dependent increase in blood molybdenum concentration in exposed mice. There were no toxicologically significant differences in bone density or curvature between control and exposed mice.

Pathology Findings

The incidences of alveolar/bronchiolar carcinoma in all exposed groups of males were significantly greater than that in the control group. Incidences of alveolar/bronchiolar adenoma in females in the 30 and 100 mg/m^3 groups were significantly greater than that in the control group. Incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 10 and 30 mg/m^3 males and in 100 mg/m^3 females were significantly greater than those in the control groups and exceeded the historical control ranges for 2-year NTP inhalation studies.

Incidences of metaplasia of the alveolar epithelium of minimal severity in the centriacinar region of the lung were significantly increased in all exposed groups of mice. The incidences of histiocyte cellular infiltration in all exposed groups of males were significantly greater than that in the control group. Incidences of hyaline degeneration of the respiratory epithelium of the nasal cavity in 100 mg/m^3 males and females and hyaline degeneration of the olfactory epithelium of the nasal cavity in 100 mg/m^3 females were significantly greater than those in the control groups. The incidences of squamous metaplasia of the epithelium lining the base of the epiglottis were significantly increased in all exposed groups of males and females. In both male and female mice, the incidences of hyperplasia of the laryngeal epithelium in level II of the larynx increased with increasing exposure concentration. The increase was statistically significant only in mice exposed to 100 mg/m^3 with 82% of male and 70% of female mice affected.

GENETIC TOXICOLOGY

Molybdenum trioxide was not mutagenic in any of five strains of *Salmonella typhimurium*, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells *in vitro*. All tests were conducted with and without S9 metabolic activation enzymes.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity** of molybdenum trioxide in male F344/N rats based on a marginally significant positive trend of alveolar/bronchiolar adenoma or carcinoma (combined). There was *no evidence of carcinogenic activity* of molybdenum trioxide in female F344/N rats exposed to 10, 30, or 100 mg/m^3. There was *some evidence of carcinogenic activity* of molybdenum trioxide in male B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar carcinoma and adenoma or carcinoma (combined). There was *some evidence of carcinogenic activity* of molybdenum trioxide in female B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined).

Exposure of male and female rats to molybdenum trioxide by inhalation resulted in increased incidences of chronic alveolar inflammation, hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), and squamous metaplasia of the epiglottis.

Exposure of male and female mice to molybdenum trioxide by inhalation resulted in increased incidences of metaplasia of the alveolar epithelium, histiocyte cellular infiltration (males), hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), squamous metaplasia of the epiglottis, and hyperplasia of the larynx.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Molybdenum Trioxide

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3
Body weights	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups greater than control group
2-Year survival rates	17/50, 10/50, 16/50, 17/50	28/50, 24/50, 24/50, 23/50	36/50, 33/50, 25/50, 37/50	25/50, 31/50, 33/50, 35/50
Nonneoplastic effects	Lung: chronic inflammation, alveolus (2/50, 3/50, 25/50, 47/50) Nose: hyaline degeneration, respiratory epithelium (2/50, 7/49, 48/49, 49/50) Larynx: squamous metaplasia, epiglottis (0/49, 11/48, 16/49, 39/49)	Lung: chronic inflammation, alveolus (14/50, 13/50, 43/50, 49/50) Nose: hyaline degeneration, respiratory epithelium (1/48, 13/49, 50/50, 50/50); hyaline degeneration, olfactory epithelium (39/48, 47/49, 50/50, 50/50) Larynx: squamous metaplasia, epiglottis (0/49, 18/49, 29/49, 49/50)	Lung: metaplasia, alveolar epithelium (0/50, 32/50, 36/49, 49/50); histiocyte infiltration, cellular (2/50, 16/50, 9/49, 9/50) Nose: hyaline degeneration, respiratory epithelium (11/50, 13/50, 11/49, 41/50) Larynx: squamous metaplasia, epiglottis (0/50, 26/49, 37/48, 49/50); hyperplasia (1/50, 3/49, 6/48, 41/50)	Lung: metaplasia, alveolar epithelium (2/50, 26/50, 39/49, 46/49) Nose: hyaline degeneration, respiratory epithelium (26/49, 23/50, 28/49, 48/49); hyaline degeneration, olfactory epithelium (22/49, 14/50, 14/49, 36/49) Larynx: squamous metaplasia, epiglottis (1/49, 36/50, 43/49, 49/50); hyperplasia (1/49, 1/50, 7/49, 35/50)
Neoplastic effects	None	None	Lung: alveolar/ bronchiolar carcinoma (2/50, 16/50, 14/49, 10/50); alveolar/ bronchiolar adenoma or carcinoma (11/50, 27/50, 21/49, 18/50)	Lung: alveolar/ bronchiolar adenoma (1/50, 4/50, 8/49, 9/49); alveolar/bronchiolar adenoma or carcinoma (3/50, 6/50, 8/49, 15/49)
Uncertain findings	Lung: alveolar/ bronchiolar adenoma (0/50, 0/50, 0/50, 3/50); alveolar/bronchiolar carcinoma (0/50, 1/50, 1/50, 1/50); alveolar/ bronchiolar adenoma or carcinoma (0/50, 1/50, 1/50, 4/50)	None	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence	Some evidence	Some evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA97, TA98, TA100, TA1535, and TA1537
Sister chromatid exchanges Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on molybdenum trioxide on 5 December 1995, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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National Cancer Institute
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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 5 December 1995, the draft Technical Report on the toxicology and carcinogenesis studies of molybdenum trioxide received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of molybdenum trioxide by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies in rats and mice were *equivocal evidence of carcinogenic activity* in male F344/N rats, *no evidence of carcinogenic activity* in female F344/N rats, and *some evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Taylor, a principal reviewer, agreed with the proposed conclusions. His major criticism concerned the failure to select higher doses for the 2-year study. While some of the outcomes, e.g., neoplasm promoter activity, were detected at doses used, toxicities and neoplasms at other sites might have been induced by doses as high as 200 mg/m^3. Dr. Chan said the dose selection was based on body weight effects in the 14-day and 13-week studies but agreed that the top dose could have been higher. Dr. Taylor noted a statement in the report that male rats exhibited higher blood molybdenum concentrations and greater variability than females. He wondered if this was related to body weight or if pharmacokinetic data were not available and there was no explanation for the blood concentration differences.

Dr. Russo, the second principal reviewer, agreed with the proposed conclusions. She inquired as to the criteria used to designate the lung neoplasm findings in male rats as "uncertain neoplastic effects." Dr. J.R. Bucher, NIEHS, said that in the summary table in the Abstract, equivocal responses were generally listed under "uncertain findings." These were neoplastic responses that may or may not be chemical-related, and they should not be discounted.

Dr. Brown initiated a discussion about the severity grades assigned to the nonneoplastic lesions of the respiratory system in rats and mice. The severity grades range from 1 (minimal) to 4 (marked). Dr. Brown noted that many of the lesions were listed as 2 (mild) or below and wondered about the significance of such apparently slight changes from normal. Dr. Carlson said he would find the inclusion of ranges of severity within a group to be helpful. Dr. J.R. Hailey, NIEHS, said lesions probably would be characterized as minor. Dr. J.K. Haseman, NIEHS, said reporting the average severity grade primarily was a space-saving device, and for selected lesions the complete distribution could be included. Dr. LeBoeuf asked why the level of evidence for male mice was not *clear evidence*, since malignant lung neoplasms at all three exposure concentrations were significantly increased over controls. Dr. Haseman said there were two reasons: (1) the lack of a dose response, and (2) for lung neoplasms, combined neoplasm incidence was given primary emphasis, so that when adenomas and carcinomas were combined, the high dose group lost significance.

Dr. G. Van Riper, Vice President, Environmental Services, Behre Dolbear & Company, Inc., said he was representing Climax Molybdenum Company, which is the largest producer of molybdenum compounds in the United States, having produced such compounds since the early 1900s. He stated that during that time there has never been correlation of cancer with employees exposed to the compounds and because of the process used, worker exposure is quite low. Dr. Van Riper said he believed the pure oxide was used in NTP studies and detailed the synthetic processes to its production. With regard to toxicity, he commented that the pure trioxide is quite acidic (pH of 3.5) and speculated that bronchial effects might be an artifact of the low pH. Dr. Van Riper concluded by referring to reports of the anticarcinogenic effects of molybdenum and the need for more discussion of such effects. Dr. Brown asked if there was any comment on effects of inhalation of other materials with such a low pH. Dr. Bucher said there have been epidemiology studies of certain refinery populations where there had been fairly high sulfuric acid levels in the air, and there was an association with increased lung cancer incidence

among the workers. Dr. Brown asked for any comment on the purported anticarcinogenic effects. Dr. Bucher said selenium was another example whereby low levels appeared to be anticarcinogenic and high levels carcinogenic. Dr. LeBoeuf agreed and said that where such data might be used in the risk assessment process, considerations of discontinuity in terms of dose and bio-logical activity needed to be discussed.

Dr. Taylor moved the Technical Report on molybdenum trioxide be accepted with revisions discussed and with the conclusions as written for male rats, *equivocal evidence of carcinogenic activity,* for female rats, *no evidence of carcinogenic activity,* and for male and female mice, *some evidence of carcinogenic activity.* Dr. Russo seconded the motion, which was accepted unanimously with seven votes.

INTRODUCTION

MoO_3

MOLYBDENUM TRIOXIDE

CAS No. 1313-27-5

Chemical Formula: MoO_3 Molecular Weight: 143.95

Synonyms: Molybdena; molybdenum anhydride; molybdenum (VI) oxide; molybdenum peroxide; molybdic acid anhydride; molybdic anhydride; molybdic oxide; molybdic trioxide; natural molybdite

CHEMICAL AND PHYSICAL PROPERTIES

Molybdenum trioxide is a white or slightly yellow to slightly bluish powder with a boiling point of 1155° C, a melting point of 795° C, and a specific gravity of 4.50 at 19.5° C. It is soluble in water (0.49 g/L at 28° C), concentrated mineral acids, and solutions of alkali hydroxides, ammonia, and potassium bitartrate. Its vapor pressure is less than 10^{-3} mm Hg at 600° C (*Merck Index*, 1989).

BIOLOGICAL FUNCTION

Molybdenum is an essential element in plants and animals. The metal is required for the function of the nitrogen-fixing enzyme, nitrogenase, in nitrogen-fixing bacteria and plants. It is a cofactor for the enzymes xanthine oxidoreductase, sulfide oxidase, and aldehyde oxidase in mammals (USEPA, 1975). Xanthine oxidoreductase is the product of a single gene and is present in two interconvertible forms, xanthine dehydrogenase and xanthine oxidase. Xanthine oxidase oxidizes hypoxanthine to xanthine and xanthine to uric acid in purine catabolism and is inhibited by the drug allopurinol. The enzyme transfers electrons from the substrate to molecular oxygen and, in the process, generates superoxide anion radicals which may cause tissue damage in many pathological conditions (Fried *et al.*, 1973; McCord, 1985). Cofactor deficiency leads to abnormal sulfur and xanthine metabolism, i.e., high xanthine and low cystine and uric acid concentrations in plasma and urine (Van Gennip *et al.*, 1994).

Sulfite oxidase catalyzes the oxidation of sulfite to sulfate, and aldehyde oxidase catalyzes the oxidation of aldehydes and various nitrogen-containing aromatic heterocyclic compounds. There is evidence that high molybdenum intake reduces dental caries in humans and animals by increasing the availability of fluoride ions (USEPA, 1975).

PRODUCTION, USE, AND HUMAN EXPOSURE

Molybdenum is widely distributed in nature. It is found in the minerals molybdenite, wulfenite, ferrimolybdate, jordisite, and powellite. It is present at an average concentration of 12 to 16 ppm in sea water and 1 ppm in the earth's crust. Molybdenum concentrations vary from 0.28 to 15 µg/g in coal from different parts of the United States (USEPA, 1975). Molybdenum concentrations are below 0.1 µg/g in

light oils and up to 0.52 µg/g in heavier oils. Due to its high boiling point, a large amount of molybdenum remains in ash after combustion (USEPA, 1975). The average concentration of molybdenum is 0.35 µg/L in large United States rivers, 1.4 µg/L (highest concentration 68 µg/L) in the drinking water of the 100 largest United States cities, and 0.005 µg/m^3 (highest concentration 0.78 µg/m^3) in urban air (Hammond and Beliles, 1980). In the Climax area of Colorado where mining is extensive, molybdenum concentrations are as high as several mg/L in water, 530 mg/kg dry weight in the river sediments, and 200 to 400 µg/L in the Dillon reservoir (USEPA, 1975).

Molybdenum occurs in five valence states: +2, +3, +4, +5, and +6. Molybdenum forms two series of stable and water-soluble salts in tri- and hexavalent states. The biological differences with respect to valence are not clear. Molybdenum trioxide is a hexavalent compound (*Kirk-Othmer*, 1981; Goyer, 1991).

Molybdenum trioxide is produced by roasting an ore containing molybdenum at 1200° F. Of all the molybdenum compounds examined, molybdenum trioxide has the largest production volume (4.4×10^{10} g in 1977). Total United States production in 1979 was 110 million pounds. Current import and export figures are not available. Molybdenum trioxide is used primarily (90% or more) as an additive to steel and corrosion-resistant alloys. It is also used as a chemical intermediate for molybdenum products; an industrial catalyst; a pigment; a crop nutrient; a component of glass, ceramics, and enamels; a flame retardant for polyester and polyvinyl chloride resins; and a reagent in chemical analyses (*Kirk-Othmer*, 1981; NIOSH/OSHA, 1981; *Patty's*, 1981).

Occupational standards of exposure established by the Occupational Safety and Health Administration (OSHA) are 5 mg/m^3 for soluble molybdenum compounds and 15 mg/m^3 for insoluble molybdenum compounds (Hammond and Beliles, 1980). In the United Kingdom the short-term exposure limit values are 10 mg/m^3 for soluble molybdenum compounds and 20 mg/m^3 for insoluble molybdenum compounds (Sittig, 1994). According to the National Occupational Exposure Survey, approximately 17,072 workers in the United States were potentially exposed to molybdenum trioxide during the years 1981 to 1983 (NIOSH, 1990). The American Conference of Governmental Industrial Hygienists (ACGIH, 1995) recommends a threshold limit value-time-weighted average of 5 mg/m^3 for soluble molybdenum compounds and 10 mg/m^3 for insoluble molybdenum.

Environmental release of molybdenum trioxide from industrial activities can occur in air (stack emissions), water (liquid effluents), or solid wastes (sludge). However, exposure of the general public to molybdenum trioxide is generally minimal because of government regulations, and the price of molybdenum trioxide (about $10 per pound) encourages efficient capture of the chemical during its production and use (Hazard Information Review, 1981). Nevertheless, release of molybdenum trioxide dust during control equipment failure has been reported. The use of molybdenum in fertilizers may also be a problem in some areas. Thus, molybdenum is a potential pollutant. It is harmful to aquatic life in very low concentrations. Human exposure to molybdenum trioxide occurs primarily through inhalation of dust and ingestion in food and drinking water. A fume hazard may result from the sublimation characteristic of molybdenum trioxide at temperatures above 800° C (*Patty's*, 1981).

Molybdenum is ubiquitous in foodstuffs and in plant and animal tissues. Shellfish have high concentrations of molybdenum because the plankton they eat concentrate the element from sea water. Humans ingest an average of 350 µg molybdenum per day in food (Hammond and Beliles, 1980). The chemical form in which molybdenum exists in plant and animal tissues is unknown (De Renzo, 1962). The daily requirement of molybdenum for humans is estimated to be 0.1 to 0.5 mg, but exact requirements are not known (Venugopal and Luckey, 1978; National Research Council, 1980). The average 70-kg man has a body content of about 9 mg molybdenum, most of it concentrated in bone, liver, kidneys, adrenal glands, and omentum. Molybdenum concentrations in newborn humans are relatively low, but the concentrations gradually increase up to age 20 and decline thereafter (Hammond and Beliles, 1980).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Molybdenum trioxide is water soluble and is absorbed rapidly in most species after oral administration, intramuscular injection, or inhalation exposure. After temporary retention in the tissues, molybdenum is excreted completely in the form of molybdate, with no accumulation in mammals. The biologic half-life is on the order of hours in experimental animals and weeks in humans (Friberg, 1979).

Low concentrations of molybdenum (20-270 µg/10 g fresh tissue) were found in the lungs, liver, kidneys, spleen, and bone of guinea pigs exposed by inhalation to the dust or fumes of molybdenum trioxide (150-300 mg/m^3) for 1 hour per day, 5 times per week, for 5 weeks (Fairhall *et al.*, 1945). The molyb-denum concentrations in these tissues gradually de-creased after molybdenum trioxide exposure was stopped; the concentration declined to about 20% of the original levels after 2 weeks.

After an oral gavage dose of 50 mg molybdenum trioxide was administered to guinea pigs, molybdenum was distributed to the kidneys, spleen, blood, bile, liver, and lungs within 4 hours. The concentrations of molybdenum in the organs decreased, whereas in the blood and bile molybdenum titers were higher at 48 hours. Bone retained molybdenum longer than any other tissue (Fairhall *et al.*, 1945). Based on the amount recovered in feces for up to 48 hours, Fairhall *et al.* (1945) calculated that 85% of the oral dose was absorbed.

Fischer rats fed dietary sodium molybdate (2 ppm) and subcutaneously injected with *N*-methyl-*N*-benzyl-nitrosamine had increased molybdenum concentrations in the esophagus, forestomach, blood serum, and liver. Xanthine oxidase activities were also increased in the esophagus and forestomach, but not in the liver (Komada *et al.*, 1990). Radioactivity was detected in the liver, bone, heart, lungs, blood, and kidneys 2.5 hours after rats were fed a single dose (13.34 mg) of ^{99}Mo. The concentrations of radioactivity were higher in the intestine, kidney, and bone than in other tissues 51 hours after dosing. It was estimated that 35% of the administered dose was absorbed (Neilands *et al.*, 1948). In dogs receiving ^{99}Mo by injection, molybdenum was selectively concentrated in the liver, kidneys, and endocrine glands (pancreas, pituitary, adrenal, and thyroid). The brain, white marrow, and fat contained negligible amounts of the injected molybdenum (*Patty's*, 1981). In lactating goats, $^{99}MoO_3$ administered orally was found in skeleton, liver, skin, muscles, blood, kidney, ovary, and hair 4 days later (Anke *et al.*, 1971).

In normal blood, molybdenum is firmly bound to red blood cells and plasma protein, with somewhat greater amounts associated with red blood cells (Bela and Lifshits, 1966; Mills and Davis, 1987).

Excessive hexavalent forms of molybdenum are excreted rapidly through the kidneys and the bile. Twice as much molybdenum is eliminated in urine as in feces. The urinary and fecal concentrations of molybdenum returned to normal 96 hours after an oral dose of molybdenum trioxide was administered to guinea pigs (Fairhall *et al.*, 1945). The predominant urinary metabolite of molybdenum was in the form of conjugated molybdate complexes (Venugopal and Luckey, 1978). Molybdenum was also detected in the milk of goats fed molybdenum trioxide (Anke *et al.*, 1971), and the concentration of molybdenum in cows' milk was increased after daily feeding of 500 mg ammonium molybdate (Mills and Davis, 1987). The absorption, tissue distribution, and excretion patterns of molybdenum in rabbits are similar to those in other species described above.

Increased molybdenum intake by experimental animals has been shown to increase tissue levels of xanthine oxidase (liver, intestine, and kidney; Luo *et al.*, 1983). Exposure to molybdenum trioxide dust (30 mg/m^3) for 5.5 months increased serum and urinary ascorbic acid levels in rabbits, but no similar effects occurred in rats (Lukashev and Shishkova, 1971a).

In mammals, there are metabolic interactions between molybdenum and copper, sulfate, and tungsten. High dietary levels of molybdenum produce a conditioned copper deficiency by depleting copper storage in the liver. Dairy cows given feed containing sodium molybdate (53-300 ppm) had decreased liver copper and increased milk copper concentrations (Huber *et al.*, 1971). Molybdenum causes an impairment of

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copper uptake by liver cells, and thus disturbs the synthesis of copper-containing proteins, including ceruloplasmin (Marcilese *et al.*, 1969). The antagonism of copper depends on sulfate concentration in the diet.

Molybdenum depresses liver sulfide oxidase activity (Halverson *et al.*, 1960). The resulting sulfide accumulation leads to the formation of highly insoluble cupric sulfide and the subsequent appearance of symptoms of copper deficiency. However, copper prevents the accumulation of molybdenum in the liver by antagonizing the absorption of molybdenum. Sulfate limits molybdenum retention both by reducing its gastrointestinal absorption and by increasing its urinary and fecal excretion. The transport of molybdenum across tissue membrane is prevented by excessive SO_4 ions (Venugopal and Luckey, 1978). Sulfate may also displace molybdate in the body. Thus copper, sulfate, and copper sulfate have been used to treat diseases caused by excessive molyb-denum (Arrington and Davis, 1953).

Tungsten is antagonistic to molybdenum. It interferes with absorption and increases urinary excretion of molybdenum. The activities of molybdenum-dependent enzymes are inhibited in neonates when pregnant animals are fed tungsten. Tungsten is believed to replace molybdate in the molybdate-dependent enzymes (De Renzo, 1962). As a result, sulfite oxidase and xanthine oxidase activities are reduced (Cohen *et al.*, 1973).

Humans

The absorption, tissue distribution, and excretion patterns of molybdenum in humans are similar to those in other species described above. As in experimental animals, high dietary levels of molybdenum produces a conditioned copper deficiency in humans. Increased urinary copper excretion and elevated levels of plasma copper were found in human volunteers ingesting 1.54 mg of molybdenum daily (Deosthale and Gopalan, 1974).

TOXICITY

Experimental Animals

Species differences exist with respect to the toxicity of molybdenum and its compounds. Cattle are the most susceptible to molybdenum toxicity, followed by sheep, guinea pigs, poultry, rats, rabbits, pigs, and horses (Davis, 1950; Miller and Engel, 1960). Molybdenum is more toxic to ruminants than to monogastric animals, probably due to the formation of thiomolybdate in the sulfide-rich environment of the rumen (Suttle, 1974; Dick *et al.*, 1975). The inhalation LC_{75} for rats is 431 mg/m^3 per hour, whereas the oral LD_{50} is 125 mg/kg (Sax, 1984). The subcutaneous LD_{50} for mice is 94 mg/kg (Sax, 1984). Acute symptoms of molybdenum toxicity include diarrhea, coma, and death from cardiac failure.

Typical symptoms of toxicity include weight loss or growth retardation, anorexia, anemia, diarrhea, achromotrichia, testicular degeneration, poor conception and deficient lactation, dyspnea, incoordination, and irritation of mucous membranes. Molybdenum also disturbs bone metabolism, giving rise to lameness, bone joint abnormalities, osteoporosis, and high serum phosphatase levels. Elevated molybdenum intake depresses copper availability and produces copper deficiency; the symptoms of molybdenosis described above are similar to those of hypocuprosis (Miller and Engel, 1960).

Cattle exposed for short periods (as little as several days) to pastures having concentrations of molybdenum in the soil and herbage that are higher (20-100 ppm) than the normal 3 to 5 ppm develop the disease known as “teart” (Lewis, 1943; Mills and Davis, 1987). The symptoms of teart include weight loss, diarrhea, loss of coat color (greying of black hair areas), anemia, poor conception and deficient lactation, lack of libido, testicular abnormalities (aspermatogenesis, interstitial cell and germinal epithelial damage), bone and joint abnormalities (brittleness and osteoporosis), and death (Thomas and Moss, 1951). Supplementing the diet with ammonium molybdate reduced serum and pituitary luteinizing hormone concentrations in dairy steers (Xin *et al.*, 1993). The poor reproductive function may be due to an effect on the hypothalamo-pituitary-gonad axis as a result of copper depletion following high molybdenum intake.

Sheep also develop the teart syndrome but are less susceptible than cattle (Mills and Davis, 1987).

Exostoses, hemorrhages about the long bones, and loosening of the great trochanters of the femurs have been described (Pitt *et al.*, 1980). The lesions were due to defects in the connective tissue at points where muscle is inserted into bone and to defects in the epiphysial plates of the trochanters.

Guinea pigs have been studied by Fairhall *et al.* (1945) by the inhalation and oral routes of administration. Inhalation exposure of 24 animals to molybdenum trioxide dust (205 mg/m^3) for 1 hour per day, 5 days per week, for 5 weeks was reported to produce eye and nasal irritation, anorexia and weight loss, diarrhea, muscular incoordination, and hair loss. Gross autopsy examination revealed changes in the liver (vacuolization and necrosis), spleen (cell damage), and lungs (alveolar and bronchiolar exudate). A 51% mortality rate was observed. Exposure to the fumes of molybdenum trioxide (191 mg/m^3) for the same duration was less toxic, with a mortality rate of only 8.3%. Oral administration by intubation of 25 to 200 mg molybdenum trioxide per guinea pig daily for 1 month produced dose-related changes in the liver (fatty changes) and kidney (focal necrosis and granulomatosis).

Numerous studies have been performed in rats. Inhalation exposure to 3 to 10 mg/m^3 molybdenum trioxide dust for 2 hours on alternate days for 2 months caused changes in the liver (dystrophic changes manifested by protoplasmic swelling and granulation, focal fatty degeneration, and binucleate cells with hyperchromic nuclei), kidney (dystrophic), and lungs (alveolar wall thickening, interstitial pneumonia, and areas of emphysema) (Russian study cited in Hazard Information Review, 1981; details unavailable). Molybdenum trioxide dust also irritates the eyes and mucous membranes. In other studies, inhalation exposure to 30 mg/m^3 molybdenum trioxide dust for 4 hours per day for 4 to 6 months (usually 5.5 months) resulted in reductions in alkaline and acid phosphatase activities in serum and decreased inorganic phosphorus levels in the tibia, but no changes were detected in ascorbic acid levels in serum or urine or in liver or spleen weights (Lukashev and Shishkova, 1971a,b,c).

Sodium molybdate administered in feed to Long-Evans rats for 4 to 7 weeks induced achromotrichia (loss of hair pigment) at 80 to 140 ppm (Jeter and Davis, 1954), femorotibial joint enlargement (epiphyseal thickening of femur characterized as chondrodystrophy of epiphyseal cartilages and mast cell accumulation in the diaphysis of long bones) at 75 to 300 ppm (Miller *et al.*, 1956), mandibular and maxillary exostoses at 400 ppm (Van Reen, 1959; Ostrom *et al.*, 1961), and diarrhea at 800 to 1,400 ppm (Ostrom *et al.*, 1961). Reduced erythrocyte counts and hemoglobin concentrations were observed at 75 to 400 ppm (Miller *et al.*, 1956; Ostrom *et al.*, 1961). Feed consumption and body weight gain were reduced at 400 to 1,200 ppm. Doses of 400 to 1,200 ppm also increased liver xanthine oxidase (Luo *et al.*, 1983) and alkaline phosphatase activities (Mills *et al.*, 1958; Van Reen, 1959); decreased liver sulfide oxidase, alkaline phosphatase, and cytochrome oxidase activities (Mills and Davis, 1987); and caused fatty degeneration of the liver and kidney (Van Reen, 1959). Deaths occurred at 4,000 to 5,000 ppm (Neilands *et al.*, 1948).

Oral intubation with a molybdenum salt, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, in water at 80 mg/kg daily for 8 weeks caused a reduction in body weight and absolute kidney weight but an increase in relative kidney weight in male Sprague-Dawley rats. The molybdenum salt induced a significant increase in diuresis and creatinuria. Glomerular function, as measured by creatinine clearance, was significantly decreased. Urinary excretion of kallikrein was increased. The renal changes were considered to indicate a mild chronic renal failure (Bompart *et al.*, 1990).

Weanling male rats fed a diet containing 6 mg molybdenum in the form of ammonium tetrathiomolybdate and 3 mg copper per kg body weight for 2 to 21 days showed severe changes at long bone growth plates, at muscle insertions, and beneath the periosteum (Spence *et al.*, 1980). Thickening and widening of the epiphyseal growth plates of the femur, tibia, humerus, radius, and ulna were observed. Microscopically, the lesions included growth plate cartilaginous dysplasia with subsequent interference with endochondral ossification, subperiosteal multiplication of osteogenic cells and production of

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large amounts of disorganized bone, resorption of trabecular bone, and interference with fibrogenesis at ligamentous attachments to bone. No toxic effects of molybdenum in mice have been reported in the literature.

Rabbits have been studied using both inhalation and oral routes of administration. In three inhalation studies, rabbits were exposed to an average chamber concentration of 30 mg/m^3 molybdenum trioxide dust for 4 hours per day for 5.5 months. Reductions in serum alkaline and acid phosphatase activities, decreased inorganic phosphorus levels in tibia, and increased ascorbic acid levels in serum and urine were reported. There were no changes in liver or spleen weights (Lukashev and Shishkova, 1971a,b,c). In an oral study in which sodium molybdate was administered in feed to weanling and adult rabbits for 5 weeks, concentrations of 0.1% to 0.4% produced anorexia, weight loss, decreased erythrocyte counts and hemoglobin concentrations, alopecia, dermatosis, and death. In addition, weanlings developed an abnormality of the front legs, i.e., an inability to support their weight, and bending of the humerus (Arrington and Davis, 1953). Administration of 5 mg/kg ammonium molybdate per day for 4 to 6 months produced an increase in spleen weight and a reduction in liver weight (Lukashev and Shishkova, 1971c).

No signs of toxicity were observed in pigs fed high levels of molybdenum (100 ppm) for 3 months (Davis, 1950) or in horses exposed to teart pastures (Lewis, 1943).

Humans

No specific symptoms of molybdenum trioxide exposure were reported in the literature except for arthralgia (pain in joints). Serum uric acid levels were increased in 34 of 37 copper-molybdenum plant workers who complained of arthralgia (USEPA, 1975).

In a plant producing molybdenum trioxide, the 8-hour exposure to respirable dusts of molybdenum trioxide and other soluble oxides of molybdenum was 9.47 mg/m^3, about twice that of the OSHA permissible exposure limit of 5 mg/m^3. Mean serum uric acid levels of 25 male workers were 1.18-fold higher ($P<0.025$) and mean serum ceruloplasmin (copper transport protein) levels were 1.65-fold higher ($P<0.005$) than those of unexposed workers. No evidence of a gout-like syndrome was observed (Walravens *et al.*, 1979). However, development of gout and multiple sclerosis has been reported in humans exposed to high molybdenum concentrations in food and air (Bruin, 1976; Pitt, 1976).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

No information on reproductive or developmental toxicity of molybdenum trioxide in experimental animals was found in the literature. However, studies of other forms of molybdenum have been performed.

In a study in which male and female rats (strain not specified) were mated after receiving 80 or 140 ppm sodium molybdate in feed from the time of weaning (approximately 3 weeks of age) to 11 weeks of age, significant findings included fewer litters, degeneration of the seminiferous tubules, and impaired growth of pups (weaning weights of the litters were reduced). The latter effect was not due to a decrease in feed consumption by any of the dams (Jeter and Davis, 1954). Significant findings in a three-generation reproduction study during which Charles River CD mice were exposed to 10 ppm molybdenum in drinking water included early deaths of the F_1 mice (15/238 exposed F_1 mice vs. 0/209 controls), the F_2 mice (7/242 exposed F_2 mice vs. 6/248 controls), and the F_3 mice (34/123 exposed F_3 mice vs. 1/230 controls). Additional findings were confined to the F_2 and F_3 mice and included failure to breed, increased maternal deaths, litters with no live pups, and the appearance of runts (11/123 exposed F_3 mice vs. 0/230 controls) (Schroeder and Mitchener, 1971).

Sodium molybdate (0.8 mg) injected into the yolk sacs of 4- and 8-day-old chick embryos failed to induce embryonic toxicity (Ridgway and Karnofsky, 1952).

When pregnant ewes were fed a diet high in molybdate, severe demyelination of the cerebral nervous system was observed in the newborn lambs (Mills and Fell, 1960). Little or no radioactivity was found in fetuses of pregnant sows sacrificed 30 hours following

an oral dose of ^{99}Mo with Na_2MoO_4 as a carrier 6 days before farrowing (Shirley *et al.*, 1954).

Humans

No information on reproductive and developmental toxicity of molybdenum trioxide or other forms of molybdenum in humans was found in a search of the available literature.

CARCINOGENICITY

Experimental Animals

Evidence of carcinogenicity of molybdenum (VI) trioxide has not been reported. However, molybdenum (III) trioxide has been reported to be weakly carcinogenic in a short-term lung adenoma assay (the Shimkin test) in strain A mice. Three groups of 20 mice were intraperitoneally injected with 50, 144, or 250 mg molybdenum (III) trioxide per kg body weight in normal saline three times per week for a total of 19 injections. The total doses received by each group were 950, 2,735, and 4,750 mg/kg. At the end of 30 weeks, the frequency of lung tumors in the 4,750 mg/kg group was significantly higher (1.13 ± 0.20 tumors per mouse in 10/15 survivors) than that in the controls (0.42 ± 0.10 tumors in 7/19 survivors). Tumor incidences of mice in the two lower dose groups were similar to the incidence in the controls (Stoner *et al.*, 1976).

Molybdenum orange (a mixture of lead chromate and lead molybdate) administered as a single 30 mg subcutaneous injection to rats produced sarcoma (rhabdomyosarcomas and fibrosarcomas) at the injection site in 36 of 40 animals after an average latency period of 32 weeks (Maltoni, 1976a,b). This study, however, is inadequate for evaluating the potential carcinogenicity of molybdenum due to the presence of lead and chromium in the test substance. These chemicals have been implicated as potential carcinogens, and lead chromate (as chromium yellow and chromium orange) also produced sarcomas in this study.

Sodium molybdate (Na_2MoO_4) administered in drinking water at a concentration of 2 mg/L reduced the incidence of *N*-nitrososarcosine ethyl ester-induced esophageal and forestomach cancer in male Sprague-Dawley rats (Luo *et al.*, 1983). Dietary molybde-num at 2 ppm significantly inhibited *N*-methyl-*N*-benzylnitrosamine-induced esophagus squamous cell carcinomas in F344 rats (Komada *et al.*, 1990). High levels of molybdenum were found in the esophagus and forestomach tissues. The incidence of mammary gland tumors induced by *N*-nitroso-*N*-methylurea (NMU) was lower in female Sprague-Dawley rats receiving 10 mg/L sodium molybdate in drinking water compared with controls (Wei *et al.*, 1985; Seaborn and Yang, 1993). Molybdenum di-chloride inhibits growth of Ehrlich ascites tumors in mice (strain not specified) (Köpf-Maier *et al.*, 1979). *N*-Nitrosodiethylamine (NDEA) is a potent rodent carcinogen, inducing liver, esophageal, forestomach, and lung tumors in mice and liver and esophageal tumors in rats (strains not specified). NDEA forms DNA adducts in rats at the O^6-guanine and O^4-thymidine positions and induces DNA strand breaks in rat liver. Sodium molybdate inhibits O^6-ethylguanine formation and prevents DNA damage in rat liver (Koizumi *et al.*, 1995). The protective action of molybdenum is considered to be enhanced detoxification by denitrosation of nitroso compounds rather than the activation reaction of dealkylation (Koizumi *et al.*, 1995). Inhibition of the NMU mammary gland carcinogenesis by sodium molybdate indicates that molybdenum acts at the promotional phase (Seaborn and Yang, 1993). Because molybdenum lowers serum copper levels and increases urinary copper excretion, molybdenum is considered to be the biological antagonist of the cancer-promoting copper (Nederbragt, 1982).

Humans

No data on the possible relationship between molyb-denum trioxide exposure and human carcinogenesis were found in the literature. However, low intake of molybdenum has been attributed to the high incidences of esophageal cancer in South Africa among the Bantu of Transkei (Burrell *et al.*, 1966), in China (Luo *et al.*, 1983), and in Russia (Nemenko *et al.*, 1976).

In contrast, Robinson and Clifford (1968) found no correlation between an above-normal incidence of nasopharyngeal carcinoma and the concentrations of food crops and soil in the high-altitude areas of Kenya.

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GENETIC TOXICITY

Little mutagenicity data are available for molybdenum trioxide. It was reported to be negative in the *Bacillus subtilis* rec assay with cold incubation (Kada *et al.*, 1980), and it was not mutagenic in any of five strains of *Salmonella typhimurium*, with or without S9 metabolic activation enzymes (Zeiger *et al.*, 1992). Additional negative results were obtained in a *Salmonella* test with molybdate orange, mixed with lead molybdate and lead chromate (Milvy and Kay, 1978). Venitt and Levy (1974) reported that various soluble salts of molybdenum (unspecified) failed to induce reversions to tryptophan prototrophy in *Escherichia coli* strains WP2, WP2*uvrA*, and WP2*exrA*.

Increased frequencies of chromosomal aberrations were reported in peripheral blood lymphocytes of workers exposed to molybdenum, molybdenite, and molybdenum trioxide (Babaian *et al*., 1980). Additional cytogenetic data for molybdenum trioxide are presented in Appendix E of this report.

STUDY RATIONALE

The NCI nominated molybdenum trioxide for toxicity and carcinogenicity studies as a representative of a group of 13 inorganic molybdenum compounds in a class study of toxicity of selected metals. Molybdenum trioxide was chosen for study because the production of molybdenum trioxide is the largest of all the molybdenum compounds examined; molybdenum trioxide has widespread industrial use, so the potential for human exposure is significant; and there is a lack of adequate test data on molybdenum compounds in general. The NTP conducted 14-day, 13-week, and 2-year inhalation studies of molybdenum trioxide in rats and mice.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF MOLYBDENUM TRIOXIDE

Molybdenum trioxide was obtained from S.W. Shattuck Chemical Company, Inc. (Houston, TX) in one lot (G1220) and from Climax Molybdenum Company (Greenwich, CT) in one lot (1104CL). Lot G1220 was used during the 14-day and 13-week studies and lot 1104CL was used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the molybdenum trioxide studies are on file at the National Institute of Environmental Health Sciences. The methods and results of these studies are detailed in Appendix K.

Each lot of the chemical, a grayish green or greenish white powdered solid, was identified as molybdenum trioxide by infrared and ultraviolet/visible spectroscopy. In addition, the identity of lot 1104CL was determined by energy dispersive X-ray analysis and X-ray diffraction. The purity of each lot was determined by elemental analyses (atomic absorption spectroscopy for lots G1220 and 1104CL and gravimetric analysis for lot G1220), Karl Fischer water analysis, and spark source mass spectrometry. In addition, the purity of lot 1104CL was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Elemental analysis by atomic absorption for molybdenum was in agreement with the theoretical value for molybdenum trioxide. Gravimetric analysis indicated a purity of 100.6% ± 0.2% for lot G1220. Karl Fischer water analysis indicated 0.03% ± 0.02% water for lot G1220 and 0.15% ± 0.03% water for lot 1104CL. Total inorganic impurities, determined by spark source mass spectrometry, were less than 3,000 ppm for lot G1220 (cadmium, ≤100 ppm; potassium, 2,400 ppm; and silicon, 180 ppm) and less than 201 ppm for lot 1104CL (sodium, 50 ppm). ICP-AES indicated a purity of 105% ± 5% for lot 1104CL relative to lot G1220, analyzed concomitantly. The overall purity for each lot was determined to be approximately 99%.

No accelerated chemical stability studies were performed for molybdenum trioxide based on literature information about physical and chemical properties of the compound (Gould, 1962; Weast, 1989). To ensure stability, the bulk chemical was stored under refrigeration when not in use and allowed to warm to room temperature overnight prior to use (14-day and 13-week studies) or stored at room temperature in 10-gallon metal drums (2-year studies). The stability of the bulk chemical was monitored periodically by the study laboratories using atomic absorption spectroscopy, gravimetric analysis (13-week studies), and ICP-AES (2-year studies). No degradation of the bulk chemical was observed.

AEROSOL GENERATION AND EXPOSURE SYSTEM

For the 14-day and 13-week studies, molybdenum trioxide was generated by Wright dust-feed mechanisms at gear ratios appropriate for each target concentration on top of approximately 1-L elutriators which opened into the top of each chamber. The airborne dust was swept into the chamber by compressor air at 30 psi and 200 L/minute. Chamber air pressure was negative with respect to that of the room.

For the 2-year studies, the molybdenum trioxide aerosol generation and delivery system was composed of four basic components: a flexible-brush dust-feed mechanism developed at the study laboratory, a Trost air impact mill, an aerosol charge neutralizer, and an aerosol distribution system (Figure K2). The Trost air impact pulverizer used the fluid energy from opposing air jets to cause particle-to-particle, head-on impaction to deagglomerate and reduce the size distribution of the feed material. Aerosol passed through the charge neutralizer and through the distribution line. At each

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chamber location, an Air-Vac pump withdrew material from the distribution line into the chamber inlet. Each distribution line branch was terminated with a high-efficiency particulate air filter to remove any excess material.

AEROSOL CONCENTRATION MONITORING

During the 14-day and 13-week studies, gravimetric samples were obtained during exposure periods from closed-face Gelman DM-450 Metricel filters in each exposure chamber two to six times per day. Samples were analyzed for molybdenum content by atomic absorption. In the 13-week studies, a real-time aerosol monitor (RAM) (Model RAM-1; GCA Corp., Bedford, MA) was used to monitor chambers in real time during the exposure periods. Readings were recorded approximately hourly for each chamber and were used to make adjustments to the dust generating systems. In the 2-year studies, molybdenum trioxide aerosol was monitored using a RAM-1 (MIE, Inc., Bedford, MA). RAMs were calibrated twice monthly. Filter samples were taken daily for gravimetric analysis of chamber concentration to verify RAM calibration.

CHAMBER ATMOSPHERE CHARACTERIZATION

Particle size distributions in each chamber were determined twice during the 14-day studies and weekly for 6 or 7 weeks then again in week 11 or 12 during the 13-week studies using an Anderson 8-stage cascade impactor with an 11-micron preseparator. Impactor samples (Mercer-style 7-stage impactor; In-Tox Products, Albuquerque, NM) were taken from each exposure chamber at monthly intervals during the 2-year studies. An estimation was made of the mass median aerodynamic particle diameter and the geometric standard deviation of each set of samples (Tables K1, K2, and K3).

For the 13-week studies, the time required to achieve 90% of target concentration at the start of exposure (T_{90}) was 23 minutes. The time required for concentration to decay to 10% of target at the end of exposure (T_{10}) was 23 minutes. For the 2-year studies, T_{90} was 7 to 13 minutes without animals present and 7 to 12 minutes with animals present. The T_{10} was 7 to 9 minutes without animals present and 9 to 10 minutes with animals present in the chambers. A T_{90} of 12 minutes was used for the 2-year studies.

Uniformity of aerosol concentration in the 2-year inhalation exposure chambers was evaluated approximately every 3 months from 12 chamber positions (one in front and one in back for each of the six possible animal cage unit positions per chamber). The means of concentration in all chambers during the 14-day studies except the 10 mg/m^3 mouse chamber were within 10% of the target concentration; the 10 mg/m^3 mouse chamber averaged 12% over target (Table K4). The means of concentration in all chambers during the 13-week studies were within 10% of the target concentration (Table K5). The means of concentration in all chambers for the 2-year studies were at least 95% of the target (Table K6). At least 82% of all concentration readings were within the specified limits.

14-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats and mice were 6 weeks old. Animals were quarantined for 2 weeks and were 8 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to molybdenum trioxide by inhalation at concentrations of 0, 3, 10, 30, 100, or 300 mg/m^3 for 6 hours per day, 5 days per week, for a total of 10 days during a 14-day period. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Rats and mice were housed individually. Clinical findings were recorded and animals were weighed initially, at one week, and at the end of the studies. A necropsy was performed on all animals. A histopathologic examination of the nose was performed on all animals. Details of the study design and animal maintenance are summarized in Table 1.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to

molybdenum trioxide and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Center (Frederick, MD). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 2 weeks and were 6 weeks old on the first day of the studies. Before initiation of the studies, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Groups of 10 male and 10 female rats and mice were exposed to molybdenum trioxide by inhalation at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 6.5 hours per day including T_{90} (23 minutes), 5 days per week, for 13 weeks. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Rats and mice were housed individually. Clinical findings were recorded weekly for rats and mice. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Clinical pathology studies were performed for all exposed and control rats at the end of the 13-week study. Rats were anesthetized and blood was drawn from the abdominal aorta. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Blood for serum analyses was collected in containers without anticoagulant, allowed to clot at room temperature, centrifuged, and the serum separated. Erythrocyte and leukocyte counts, hemoglobin concentration, and hematocrit values were determined using a Coulter S+ hematology analyzer (Coulter Electronics, Hialeah, FL). Differential leukocyte counts and morphological evaluation of blood cells were determined by light microscopic examination of blood films stained with Wright-Giemsa. Clinical chemistry determinations were performed on a CentrifiChem® chemistry analyzer (Baker Instruments, Allentown, PA). The hematology and clinical chemistry parameters evaluated are listed in Table 1. All rats and mice were evaluated for liver copper burden. Liver tissue was prepared by wet digestion with nitric and perchloric acids for copper analysis by atomic absorption spectroscopy.

At the end of the 13-week study, sperm samples were collected from 0, 10, 30, and 100 mg/m^3 rats and mice by the standard methods (NTP, 1983). The parameters evaluated are listed in Table 1. For sperm analyses, the left epididymis and testis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except testis with epididymis which was fixed in Bouin's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on 0 and 100 mg/m^3 rats and mice. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to molybdenum trioxide by inhalation at concentrations of 0, 10, 30, or 100 mg/m^3 for 6 hours

per day plus T_{90} (12 minutes), 5 days per week, for 105 (mice) or 106 (rats) weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 15 days before the beginning of the studies. Five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. Animals were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix M).

Animal Maintenance

Rats and mice were housed individually. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Cages and racks were rotated weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix L.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded initially, weekly for 12 weeks, at week 15, monthly thereafter, and at the end of the studies. Clinical observations were recorded initially, at 4, 8, 12, and 15 weeks, monthly thereafter, and at the end of the studies. Blood was collected from 10 randomly selected animals per group at the end of the studies (Appendix G). Blood molybdenum concentrations were determined with inductively coupled plasma-atomic emission spectrometry. Samples were prepared and analyzed in batches of about 40 samples each, with each batch including five quality control standards prepared by spiking blank blood or serum with approximately 0.5 µg/g molybdenum. These standards were digested and analyzed with the samples. One quality control standard was analyzed after calibration and one was analyzed after every 10 samples. The measured concentration was required to be within 20% of the known value, or the instrument was recalibrated before proceeding with further sample assays.

Right femurs were collected from 10 randomly selected animals per group at the end of the studies (Appendix J). Bone density was calculated by weighing freshly collected right femurs both in air and suspended in water. Femoral curvature was assessed by measuring two lengths defined between points on the femur tangential to a straight line placed along the medial aspect. The curvature was defined as the ratio of the length following the outline of the bone and the straight-line distance between the landmarks. Measurements were made using Bioscan Optimas image analysis software (Bioscan, Inc., Edmonds, WA).

A complete necropsy was performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist reviewed the nose, larynx, lung, and thyroid gland of rats and mice; adrenal gland, heart, kidney, and skin of male and female rats; brain, liver, pancreas, pancreatic islets, prostate gland, spleen, and unspecified tissue of male rats; clitoral gland, mammary gland, ovary, pituitary gland, and urinary bladder of female rats; epididymis and small intestine of male mice; and ovary and uterus of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues

and addressed any inconsistencies in the diagnosis made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of quality assessment pathologists, the PWG chairperson, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al*. (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al*. (1986).

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B4, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al*., 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Data for clinical chemistry, hematology, blood molybdenum levels, spermatid evaluations, liver copper levels, and bone density and curvature, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of molybdenum trioxide was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of molybdenum trioxide are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis

and the somatic mutation theory (Miller and Miller , 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical' s potential electrophilicity (structural alert to DN A reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilic-ity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicit y in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991) . Other *in vitro* genetic toxicity tests do not cor-relat e well with rodent carcin ogenicity (Tennant *et al*., 1987; Zeiger *et al*., 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chem-ica l being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcin-ogens), and that there is no complementarity among th e *in vitro* genetic toxicity tests. That is, no battery o f tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Molybdenum Trioxide

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory Hazleton Laboratories America, Inc. (Vienna, VA)	Hazleton Laboratories America, Inc. (Vienna, VA)	Battelle Pacific Northwest Laboratories (Richland, WA)
Strain and Species Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source Simonsen Laboratories (Gilroy, CA)	Frederick Cancer Research Center (Frederick, MD)	Simonsen Laboratories (Gilroy, CA)
Time Held Before Studies 2 weeks	2 weeks	15 days
Average Age When Studies Began 8 weeks	6 weeks	6 weeks
Date of First Dose Rats: 8 December 1982 Mice: 9 December 1982	Rats: 8-9 June 1983 Mice: 15-16 June 1983	Rats: 15 March 1990 Mice: 22 March 1990
Duration of Dosing 6 hours per day, 5 days per week, for 2 weeks	6.5 hours per day including T_{90} (23 minutes), 5 days per week, for 13 weeks	6 hours per day plus T_{90} (12 minutes), 5 days per week, for 105 (mice) or 106 (rats) weeks
Date of Last Dose Rats: 21 December 1982 Mice: 22 December 1982	Rats: 6-7 September 1983 Mice: 14-15 September 1983	Rats: 19 March 1992 Mice: 25 March 1992
Necropsy Dates Rats: 22 December 1982 Mice: 23 December 1982	Rats: 7-9 September 1983 Mice: 14-16 September 1983	Rats: 18-20 March 1992 Mice: 23-27 March 1992
Average Age at Necropsy 10 weeks	19 weeks	110 (mice) or 111 (rats) weeks
Size of Study Groups five males and five females	10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as 14-day studies	Same as 14-day studies
Animals per Cage 1	1	1
Method of Animal Identification Ear punch	Ear punch	Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Molybdenum Trioxide (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Diet		
NIH-07 open formula, pellets (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods	Same as 14-day studies	Same as 14-day studies, except changed weekly
Water Distribution		
Tap water (City of Vienna water supply) available *ad libitum* via automatic watering system	Same as 14-day studies	Tap water (City of Richland water supply) available *ad libitum* via an automatic watering system (Edstrom Industries, Waterford, WI), changed weekly
Cages		
Stainless steel mesh cages, changed twice weekly	Same as 14-day studies	Stainless-steel wire-bottom (Hazleton Systems, Inc., Aberdeen, MD), changed weekly
Bedding/Cageboard		
None	None	Untreated Techsorb (Shepherd Specialty Papers, Inc., Kalamazoo, MI) or untreated (Bonzl Cincinnati Paper Co., Cincinnati, OH) removed during exposure periods and changed daily
Chamber Filters		
HEPA (intake and exhaust)	HEPA (intake) and charcoal and HEPA (exhaust)	Single HEPA (Flanders Filters, Inc., San Rafael, CA); charcoal (RSE, Inc., New Baltimore, MI)
Racks		
Stainless steel	Stainless steel	Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed weekly
Animal Room Environment		
Temperature: 21°-26° C Relative humidity: 30%-71% Fluorescent light: 12 hours/day Chamber air: 200 L/minute	Temperature: 20°-31° C Relative humidity: 55%-95% Fluorescent light: 12 hours/day Chamber air: 200 L/minute	Temperature: 21°-27° C Relative humidity: 31%-84% (rats) or 28%-87% (mice) Fluorescent light: 12 hours/day Chamber air: 15 ± 3 changes/hour
Exposure Concentrations		
0, 3, 10, 30, 100, or 300 mg/m^3	0, 1, 3, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3
Type and Frequency of Observation		
Observed daily; animals were weighed and clinical findings were recorded initially, at 1 week, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly.	Observed twice daily; animals were weighed initially, weekly for 12 weeks, at week 15, monthly thereafter, and at the end of the studies. Clinical findings were recorded initially, at 4, 8, 12, and 15 weeks, monthly thereafter, and at the end of the studies.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Molybdenum Trioxide (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Method of Sacrifice		
Intraperitoneal injection of pentobarbital, followed by exsanguination	Intraperitoneal injection of pentobarbital, followed by exsanguination	Asphyxiation with 70% CO_2
Necropsy		
Necropsy performed on all animals.	Necropsy performed on all animals. Organs weighed were brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals.
Clinical Pathology		
None	Blood was collected from the abdominal aorta of rats at necropsy for hematology and clinical chemistry analyses. ***Hematology:*** hematocrit, hemoglobin, erythrocyte count, and leukocyte count and differential ***Clinical Chemistry:*** calcium and inorganic phosphorus concentrations; alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, and sorbitol dehydrogenase activities	Ten male and 10 female rats and mice were randomly selected for blood molybdenum concentration determinations.
Histopathology		
Histopathology was performed on the nose of all rats and mice.	Complete histopathology was performed on 0 and 100 mg/m^3 rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), esophagus, eye, femorotibial joint, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine, kidney, larynx, liver, lung, lymph nodes (mandibular, mediastinal, peribronchial), mammary gland, nose (all animals in all exposure groups), ovary, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, seminal vesicle (mice), spinal cord, spleen, sternum, stomach, testis and epididymis, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, femur, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung and bronchi, lymph nodes (mandibular, mesenteric, bronchial, mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach, testes and epididymis, seminal vesicle, thymus, thyroid, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Molybdenum Trioxide (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Sperm Motility		
None	At terminal sacrifice, sperm samples were collected from 0, 10, 30, and 100 mg/m^3 rats and mice. The parameters evaluated included: sperm count and motility.	None
Liver Copper Analysis		
None	Liver copper concentrations were determined from a portion of the liver taken from rats and mice at necropsy.	None
Bone Density and Curvature Determinations		
None	None	Ten male and 10 female rats and mice were randomly selected from each exposure group for bone density and curvature studies.

RESULTS

RATS

14-DAY STUDY

All rats survived to the end of the study (Table 2). The final mean body weights of male rats exposed to 100 mg/m^3 and male and female rats exposed to 300 mg/m^3 were significantly lower than those of the control groups. Male rats exposed to 300 mg/m^3 lost weight during the study. There were no clinical findings related to exposure to molybdenum trioxide. No chemical-related lesions were observed.

TABLE 2
Survival and Body Weights of Rats in the 14-Day Inhalation Study of Molybdenum Trioxide

Dose (mg/m^3)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Initial	Final	
Male				
0	5/5	158 ± 10	215 ± 8	
3	5/5	156 ± 9	208 ± 9	97
10	5/5	155 ± 11	213 ± 11	99
30	5/5	156 ± 11	215 ± 9	100
100	5/5	155 ± 12	193 ± 16*	90
300	5/5	156 ± 13	149 ± 31**	69
Female				
0	5/5	119 ± 4	143 ± 5	
3	5/5	116 ± 5	144 ± 11	101
10	5/5	115 ± 4	138 ± 13	97
30	5/5	116 ± 4	142 ± 7	99
100	5/5	117 ± 4	135 ± 4	94
300	5/5	116 ± 5	124 ± 5**	87

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard deviation.

13-WEEK STUDY

All rats survived to the end of the study (Table 3). The final mean body weights of exposed rats were similar to those of the control groups. No clinical findings related to molybdenum trioxide exposure were observed.

There were no significant differences between control and exposed rats in absolute or relative organ weights (Table F1), hematology or clinical chemistry parameters (Table G1), sperm counts or motility (Table H1), or liver copper concentrations (Table I1). No chemical-related lesions were observed.

Exposure Concentration Selection Rationale: The 13-week study did not provide adequate information on which to select the exposure concentrations for the 2-year study. However, based on lower final mean body weights of rats exposed to 300 mg/m^3 in the 14-day study, exposure concentrations selected for the 2-year study were 10, 30, and 100 mg/m^3.

TABLE 3
Survival and Body Weights of Rats in the 13-Week Inhalation Study of Molybdenum Trioxide

Dose (mg/m^3)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Initial	Final	
Male				
0	10/10	137 ± 5	327 ± 24	
1	10/10	137 ± 6	327 ± 20	100
3	10/10	137 ± 4	330 ± 18	101
10	10/10	135 ± 9	335 ± 30	102
30	10/10	137 ± 5	335 ± 16	102
100	10/10	137 ± 4	328 ± 24	100
Female				
0	10/10	108 ± 3	191 ± 6	
1	10/10	108 ± 4	190 ± 9	99
3	10/10	109 ± 3	193 ± 7	101
10	10/10	108 ± 3	182 ± 8	95
30	10/10	107 ± 3	189 ± 11	99
100	10/10	107 ± 3	191 ± 10	100

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard deviation. Differences from the control group were not significant by Williams' or Dunnett's test.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 1). Survival rates of exposed male and female rats were similar to those of the control groups.

Body Weights and Clinical Findings

Mean body weights of exposed groups of male and female rats were similar to those of the control groups throughout the study (Figure 2 and Tables 5 and 6).

No clinical findings related to molybdenum trioxide exposure were observed.

Special Studies

There was a significant exposure-dependent increase in blood molybdenum concentration in exposed rats (Table G2). Blood concentrations of molybdenum in exposed male rats were greater than those in exposed female rats. There were no toxicologically significant differences in bone density or curvature between control and exposed rats (Table J1).

TABLE 4
Survival of Rats in the 2-Year Inhalation Study of Molybdenum Trioxide

	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	29	35	31	28
Natural deaths	4	5	3	5
Animals surviving to study termination	17[d]	10	16	17
Percent probability of survival at end of study[a]	34	20	32	34
Mean survival (days)[b]	645	617	646	654
Survival analyses[c]	P=0.198N	P=0.142	P=1.000	P=0.681N
Female				
Animals initially in study	50	50	50	50
Moribund	18	25	23	23
Natural deaths	4	1	3	4
Animals surviving to study termination	28	24	24	23[d]
Percent probability of survival at end of study	56	48	48	46
Mean survival (days)	685	684	678	673
Survival analyses	P=0.328	P=0.426	P=0.459	P=0.253

[a] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[b] Mean of all deaths (censored, uncensored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or lower mortality in an exposure group is indicated by N.
[d] Includes one animal that died during the last week of the study

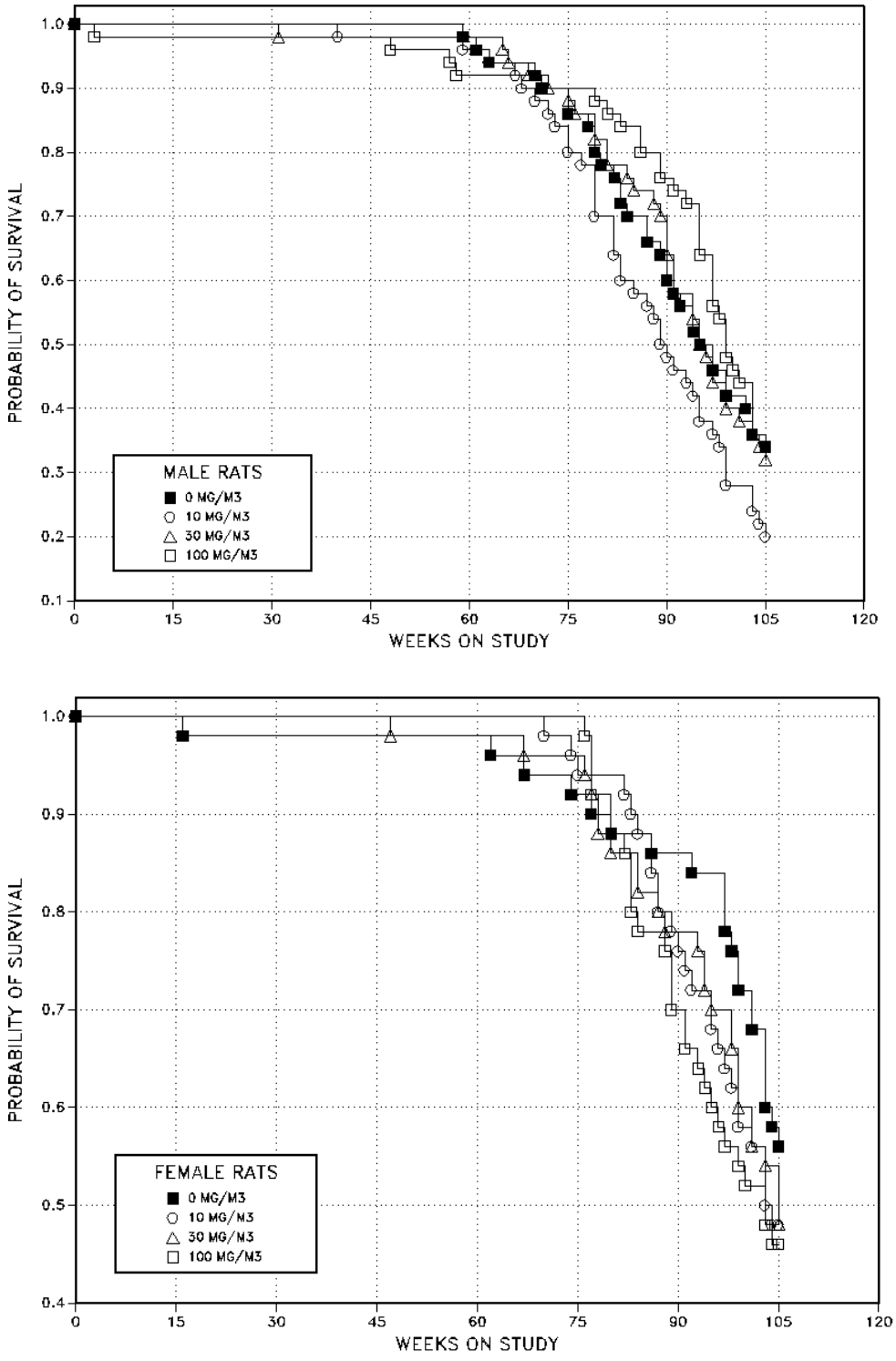


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Molybdenum Trioxide by Inhalation for 2 Years

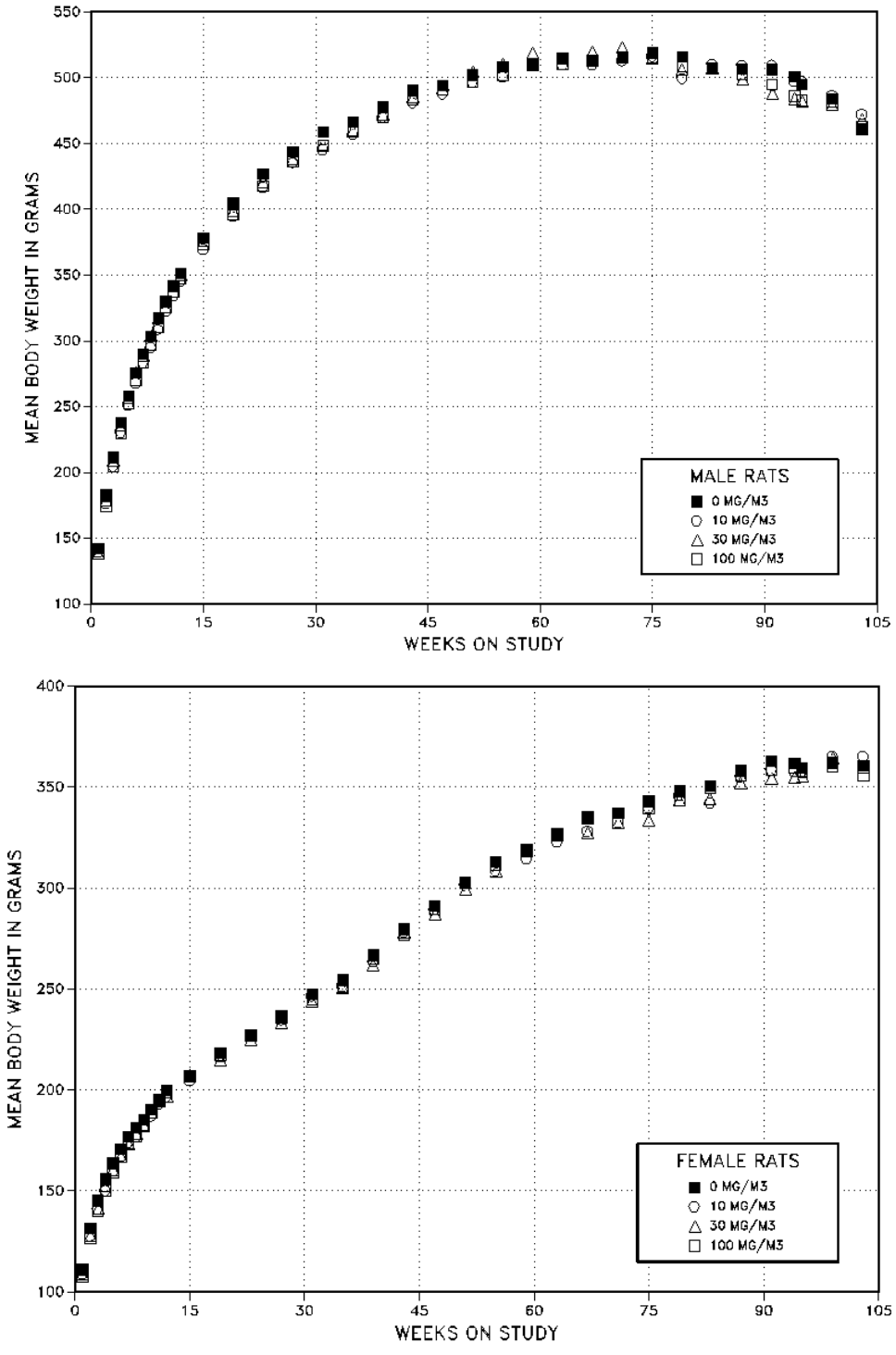


FIGURE 2
Growth Curves for Male and Female Rats
Administered Molybdenum Trioxide by Inhalation for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Molybdenum Trioxide

Weeks on Study	0 mg/m³		10 mg/m³			30 mg/m³			100 mg/m³		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	142	50	140	98	50	139	98	50	138	97	50
2	183	50	177	97	50	179	98	50	174	95	50
3	211	50	205	97	50	209	99	50	205	97	49
4	238	50	230	97	50	236	99	50	230	97	49
5	258	50	251	97	50	257	100	50	252	98	49
6	276	50	268	97	50	276	100	50	270	98	49
7	290	50	283	98	50	289	100	50	284	98	49
8	303	50	295	97	50	304	100	50	297	98	49
9	318	50	309	97	50	317	100	50	310	98	49
10	330	50	322	98	50	330	100	50	325	99	49
11	342	50	335	98	50	341	100	50	337	99	49
12	351	50	346	98	50	350	100	50	348	99	49
15	378	50	370	98	50	376	100	50	373	99	49
19	405	50	395	98	50	399	99	50	396	98	49
23	427	50	417	98	50	421	99	50	418	98	49
27	444	50	436	98	50	438	99	50	436	98	49
31	459	50	446	97	50	449	98	49	448	98	49
35	467	50	457	98	50	460	99	49	460	99	49
39	478	50	470	98	50	472	99	49	470	98	49
43	490	50	481	98	49	485	99	49	484	99	49
47	494	50	488	99	49	494	100	49	491	99	49
51	502	50	497	99	49	504	101	49	497	99	48
55	508	50	501	99	49	510	100	49	502	99	48
59	510	50	510	100	48	519	102	49	511	100	46
63	515	47	510	99	47	516	100	49	510	99	46
67	512	47	510	100	47	520	101	47	513	100	46
71	515	45	513	100	44	523	102	46	515	100	46
75	519	43	516	100	40	515	99	45	515	99	45
79	516	41	500	97	39	507	98	43	508	99	44
83	507	38	510	101	32	507	100	39	507	100	43
87	507	34	510	101	28	499	98	37	503	99	40
91	506	29	510	101	23	488	96	31	495	98	37
94	501	28	497	99	22	484	97	29	486	97	36
95	495	26	498	101	20	482	97	27	483	98	33
99	484	22	487	101	15	480	99	22	482	100	26
103	461	19	472	103	12	468	102	19	463	101	20
Mean for weeks											
1-13	270		263	97		269	100		264	98	
14-52	454		446	98		450	99		447	98	
53-103	504		503	100		501	99		500	99	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Molybdenum Trioxide

Weeks on Study	0 mg/m^3		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	111	50	109	98	50	109	98	50	107	97	50
2	132	50	128	97	50	128	97	50	127	96	50
3	145	50	141	97	50	142	98	50	140	97	50
4	156	50	150	96	50	153	98	50	150	96	50
5	164	50	160	98	50	160	98	50	159	97	50
6	171	50	167	98	50	167	98	50	167	98	50
7	177	50	174	99	50	173	98	50	173	98	50
8	181	50	177	98	50	178	98	50	177	98	50
9	185	50	182	98	50	183	99	50	182	98	50
10	190	50	187	98	50	189	99	50	189	99	50
11	195	50	193	99	50	195	100	50	195	100	50
12	200	50	197	98	50	197	98	50	199	99	50
15	207	50	205	99	50	207	100	50	207	100	50
19	218	49	217	99	50	215	98	50	217	100	50
23	227	49	227	100	50	225	99	50	227	100	50
27	236	49	235	100	50	233	99	50	237	100	50
31	247	49	247	100	50	244	99	50	245	99	50
35	255	49	251	99	50	251	99	50	251	99	50
39	267	49	264	99	50	262	98	50	265	99	50
43	280	49	278	100	50	278	99	50	277	99	50
47	291	49	289	99	50	287	99	49	290	100	50
51	303	49	302	100	50	299	99	49	303	100	50
55	313	49	309	99	50	308	99	49	312	100	50
59	319	49	315	99	50	319	100	49	319	100	50
63	327	48	323	99	50	326	100	49	327	100	50
67	334	47	328	98	50	327	98	48	335	100	50
71	337	47	332	99	49	332	99	48	337	100	50
75	343	46	340	99	48	333	97	48	340	99	50
79	348	45	346	99	47	343	99	44	343	99	46
83	351	44	342	98	45	344	98	43	350	100	41
87	358	43	355	99	41	352	98	40	356	99	39
91	363	43	359	99	38	354	98	39	358	99	34
94	362	42	358	99	36	355	98	38	360	99	31
95	359	42	357	99	34	355	99	36	358	100	30
99	362	37	365	101	30	364	101	31	360	100	27
103	361	30	365	101	28	360	100	28	356	99	25
Mean for weeks											
1-13	167		164	98		165	99		164	98	
14-52	253		252	100		250	99		252	100	
53-103	346		342	99		341	99		344	99	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the respiratory system (lung, nose, and larynx), clitoral gland, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Respiratory System: The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were increased in male rats with a marginally significant positive trend (Tables 7 and A3). However, the incidences were within the range of historical controls for 2-year NTP inhalation studies (Tables 7 and A4). No increase in the incidences of lung neoplasms occurred in female rats (Tables 7 and B3). Incidences of chronic alveolar inflammation in male and female rats exposed to 30 or 100 mg/m^3 were significantly greater than those in the control groups (Tables 7, A5, and B4). The severity of chronic inflammation was greater in rats exposed to 100 mg/m^3 compared to control rats or rats exposed to 10 or 30 mg/m^3.

Chronic inflammation in the alveoli in control rats consisted of focal intra-alveolar aggregates of a few foamy macrophages. Such aggregates are commonly observed in the alveoli of rats of various ages but are more prevalent in aged rats. Chronic inflammation in the alveoli of exposed rats was a multifocal lesion localized to subpleural and peribronchiolar sites (Plates 1 and 2). Lesions were variably sized, sharply delineated, and densely cellular consisting of aggregates of predominantly large foamy macrophages, with lesser numbers of epithelioid cells, multinucleated giant cells, and neutrophils mixed with cellular debris (Plate 3). Cholesterol crystals (clefts) were present within macrophages and multinucleated giant cells and free among the inflammatory cells in many lesions (Plate 4). Within lesions, alveolar septae were thickened by mild interstitial fibrosis, infiltrates of mononuclear inflammatory cells, and hyperplastic type II epithelial cells, many of which were vacuolated (Plates 3 and 4). Alveoli in some advanced lesions were lined by ciliated cuboidal or columnar cells (metaplasia) and frequently contained mucus (Plate 5). In the most severe lesions, fibrous tissue obliterated the alveolar architecture. Mast cells were often prominent in lesions with significant amounts of fibrous tissue.

No nasal neoplasms were attributed to exposure to molybdenum trioxide (Tables A3 and B3). Incidences of hyaline degeneration in the nasal respiratory epithelium in 30 and 100 mg/m^3 males and in all exposed groups of females were significantly greater than those in the control groups (Tables 7, A5, and B4). Most male and all female rats exposed to 30 or 100 mg/m^3 and several male and female rats exposed to 10 mg/m^3 were affected. However, the severity of the lesion was generally mild in the 100 mg/m^3 groups and minimal in the control groups and 10 and 30 mg/m^3 groups. The incidences of hyaline degeneration in the nasal olfactory epithelium of all exposed groups of females were significantly greater than in the control group.

Hyaline degeneration is a common age-related lesion in the nasal epithelium of rats. The incidence and severity of this change has been observed to increase proportionally with exposure concentration in inhalation studies and is considered a nonspecific defensive response to prolonged inhalation of a variety of irritants. Hyaline degeneration generally affected the respiratory epithelium of the nasal septum in level II of the nasal cavity and the olfactory epithelium in levels II and III of the nasal cavity. The epithelial cells in these regions contained variably sized brightly eosinophilic cytoplasmic globules which often filled and distorted the cells (Plate 6). Affected segments of the olfactory epithelium often had fewer cell layers and disorganization of the typically layered rows of cell nuclei.

No laryngeal neoplasms were attributed to exposure to molybdenum trioxide (Tables A3 and B3). Incidences of squamous metaplasia of the epithelium lining the base of the epiglottis in all exposed groups of male and female rats were significantly greater than those in the control groups and increased with increasing exposure concentration (Tables 7, A5, and B4). The severity of squamous metaplasia was generally mild at 100 mg/m^3 and minimal at 10 and 30 mg/m^3, and this lesion most likely represents a mild toxic and/or adaptive response to chronic inhalation exposure to molybdenum.

TABLE 7
Incidences of Respiratory System Neoplasms and Nonneoplastic Lesions in Rats in the 2-Year Inhalation Study of Molybdenum Trioxide

	0 mg/m^3		10 mg/m^3		30 mg/m^3		100 mg/m^3	
Male								
Nose[a]	50		49		49		50	
Respiratory Epithelium, Degeneration, Hyaline[b]	2	(1.0)[c]	7	(1.0)	48**	(1.6)	49**	(2.0)
Larynx	49		48		49		49	
Epiglottis, Metaplasia, Squamous	0		11**	(1.6)	16**	(1.7)	39**	(2.3)
Lung	50		50		50		50	
Alveolus, Inflammation, Chronic	2	(1.0)	3	(1.0)	25**	(1.5)	47**	(2.5)
Alveolar/bronchiolar Adenoma	0		0		0		3	
Alveolar/bronchiolar Carcinoma	0		1		1		1	
Alveolar/bronchiolar Adenoma or Carcinoma[d]	0		1		1		4	
Female								
Nose	48		49		50		50	
Olfactory Epithelium, Degeneration, Hyaline	39	(1.2)	47*	(1.5)	50**	(3.0)	50**	(3.2)
Respiratory Epithelium, Degeneration, Hyaline	1	(1.0)	13**	(1.2)	50**	(1.9)	50**	(2.0)
Larynx	49		49		49		50	
Epiglottis, Metaplasia, Squamous	0		18**	(1.3)	29**	(1.7)	49**	(2.2)
Lung	50		50		50		50	
Alveolus, Inflammation, Chronic	14	(1.1)	13	(1.2)	43**	(1.7)	49**	(3.0)
Alveolar/bronchiolar Adenoma	0		1		0		2	
Alveolar/bronchiolar Carcinoma	0		1		0		0	
Squamous Cell Carcinoma	1		0		0		0	
Alveolar/bronchiolar Adenoma, Alveolar/bronchiolar Carcinoma, or Squamous Cell Carcinoma	1		2		0		2	

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year NTP inhalation studies with untreated controls (mean ± standard deviation): 23/654 (3.5% ± 3.7%); range 0-10%

In affected rats, the single layer of ciliated cuboidal to columnar epithelium that normally lines the base of the epiglottis (Plate 7) was replaced by an epithelium that consisted of a basal layer of cuboidal cells with one or more superficial layers of flattened epithelial (squamous) cells that lacked cilia (Plate 8). Keratinization of the epithelial surface was common.

Clitoral Gland: The incidence of clitoral gland adenoma or carcinoma (combined) in the 30 mg/m^3 female rats was greater than that in the controls (0 mg/m^3, 3/44; 10 mg/m^3, 7/48; 30 mg/m^3, 10/47; 100 mg/m^3, 3/47; Table B3). The increase was marginally significant but not exposure concentration-related and was considered to be a spurious event unrelated to chemical exposure.

Mammary Gland: The incidence of mammary gland fibroadenoma, adenoma, or carcinoma (combined) was significantly increased in female rats exposed to 10 mg/m^3 (23/50, 33/50, 30/50, 20/50; Table B3). However, the incidences in the controls and the two lowest exposure groups exceeded the historical control range for 2-year NTP inhalation studies (202/653; mean ± standard deviation: 31% ± 9%; range: 16%-46%; includes data for fibroma, fibroadenoma, adenoma, and carcinoma) and were not exposure concentration-related and therefore not considered to be related to chemical exposure.

MICE

14-DAY STUDY

All mice survived to the end of the study (Table 8). Final mean body weights of male and female mice exposed to 300 mg/m^3 were significantly lower than those of the control groups. Male mice exposed to 300 mg/m^3 lost weight during the study. There were no clinical findings related to exposure to molybdenum trioxide. No chemical-related lesions were observed.

TABLE 8
Survival and Body Weights of Mice in the 14-Day Inhalation Study of Molybdenum Trioxide

Dose (mg/m^3)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Initial	Final	
Male				
0	5/5	24.4 ± 0.7	26.0 ± 1.0	
3	5/5	24.1 ± 0.2	25.4 ± 0.9	98
10	5/5	23.9 ± 0.7	25.6 ± 0.6	98
30	5/5	24.4 ± 0.8	25.8 ± 0.8	99
100	5/5	24.2 ± 0.8	25.1 ± 0.9	97
300	5/5	24.4 ± 1.1	23.4 ± 2.0**	90
Female				
0	5/5	20.8 ± 1.5	22.8 ± 1.1	
3	5/5	20.7 ± 1.3	22.8 ± 0.8	100
10	5/5	20.6 ± 1.2	21.1 ± 0.7	93
30	5/5	20.7 ± 1.1	21.7 ± 1.0	95
100	5/5	21.2 ± 0.8	22.2 ± 0.9	97
300	5/5	20.0 ± 0.9	20.4 ± 1.7**	89

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard deviation.

13-WEEK STUDY

All mice survived to the end of the study (Table 9). The final mean body weights of exposed mice were similar to those of the control groups. There were no chemical-related clinical findings.

There were no significant differences between control and exposed mice in absolute or relative organ weights (Table F2) or in epididymal weights, sperm counts, or motility (Table H2). There were signifi-cant increases in liver copper concentrations in female mice exposed to 30 mg/m^3 and in male and female mice exposed to 100 mg/m^3 compared to those of the control groups (Table I2). No chemical-related lesions were observed.

Exposure Concentration Selection Rationale: The 13-week study did not provide adequate information on which to select the exposure concentrations for the 2-year study. However, based on lower final mean body weights of mice exposed to 300 mg/m^3 in the 14-day study, the exposure concentrations selected for the 2-year study were 10, 30, and 100 mg/m^3.

TABLE 9
Survival and Body Weights of Mice in the 13-Week Inhalation Study of Molybdenum Trioxide

Dose (mg/m^3)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Initial	Final	
Male				
0	10/10	23.0 ± 0.9	29.8 ± 1.2	
1	10/10	22.9 ± 0.7	30.0 ± 1.3	101
3	10/10	23.0 ± 0.7	30.2 ± 0.9	101
10	10/10	23.1 ± 0.9	29.6 ± 1.1	99
30	10/10	23.3 ± 0.9	28.9 ± 1.6	97
100	10/10	23.1 ± 0.8	29.4 ± 1.4	99
Female				
0	10/10	18.6 ± 0.3	26.3 ± 1.0	
1	10/10	18.9 ± 0.7	27.3 ± 1.7	104
3	10/10	18.4 ± 0.7	26.3 ± 1.4	100
10	10/10	18.9 ± 0.7	25.7 ± 0.9	98
30	10/10	18.8 ± 0.7	25.7 ± 0.9	98
100	10/10	18.6 ± 0.8	26.3 ± 1.4	100

[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard deviation. Differences from the control group were not significant by Williams' or Dunnett's test.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 3). The survival rate of male mice exposed to 30 mg/m^3 was marginally lower than that of the control group; survival rates of 10 and 100 mg/m^3 males and of all exposed groups of females were similar to those of the control groups.

Body Weights and Clinical Findings

Mean body weights of exposed male mice were generally similar to those of the control group throughout the study (Table 11 and Figure 4). Mean body weights of exposed female mice were generally greater than those of the control group from week 11 until the end of the study (Table 12 and Figure 4). No clinical findings related to molybdenum trioxide exposure were observed.

Special Studies

There was a significant exposure-dependent increase in blood molybdenum concentration in exposed mice (Table G3). There were no toxicologically significant differences in bone density or curvature between control and exposed mice (Table J2).

TABLE 10
Survival of Mice in the 2-Year Inhalation Study of Molybdenum Trioxide

	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	8	13	14	9
Natural deaths	6	4	11	4
Animals surviving to study termination	36	33	25	37
Percent probability of survival at end of study[a]	72	66	50	74
Mean survival (days)[b]	689	706	663	701
Survival analysis[c]	P=0.536N	P=0.843	P=0.052	P=0.908N
Female				
Animals initially in study	50	50	50	50
Accidental deaths[d]	0	1	1	0
Moribund	17	12	14	10
Natural deaths	8	6	2	5
Animals surviving to study termination	25[e]	31	33[f]	35
Percent probability of survival at end of study	50	64	67	70
Mean survival (days)	683	689	704	681
Survival analysis	P=0.174N	P=0.230N	P=0.074N	P=0.096N

[a] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or lower mortality in an exposure group is indicated by N.
[d] Censored from survival analyses
[e] Includes one animal that died during the last week of the study
[f] Includes two animals that died during the last week of the study

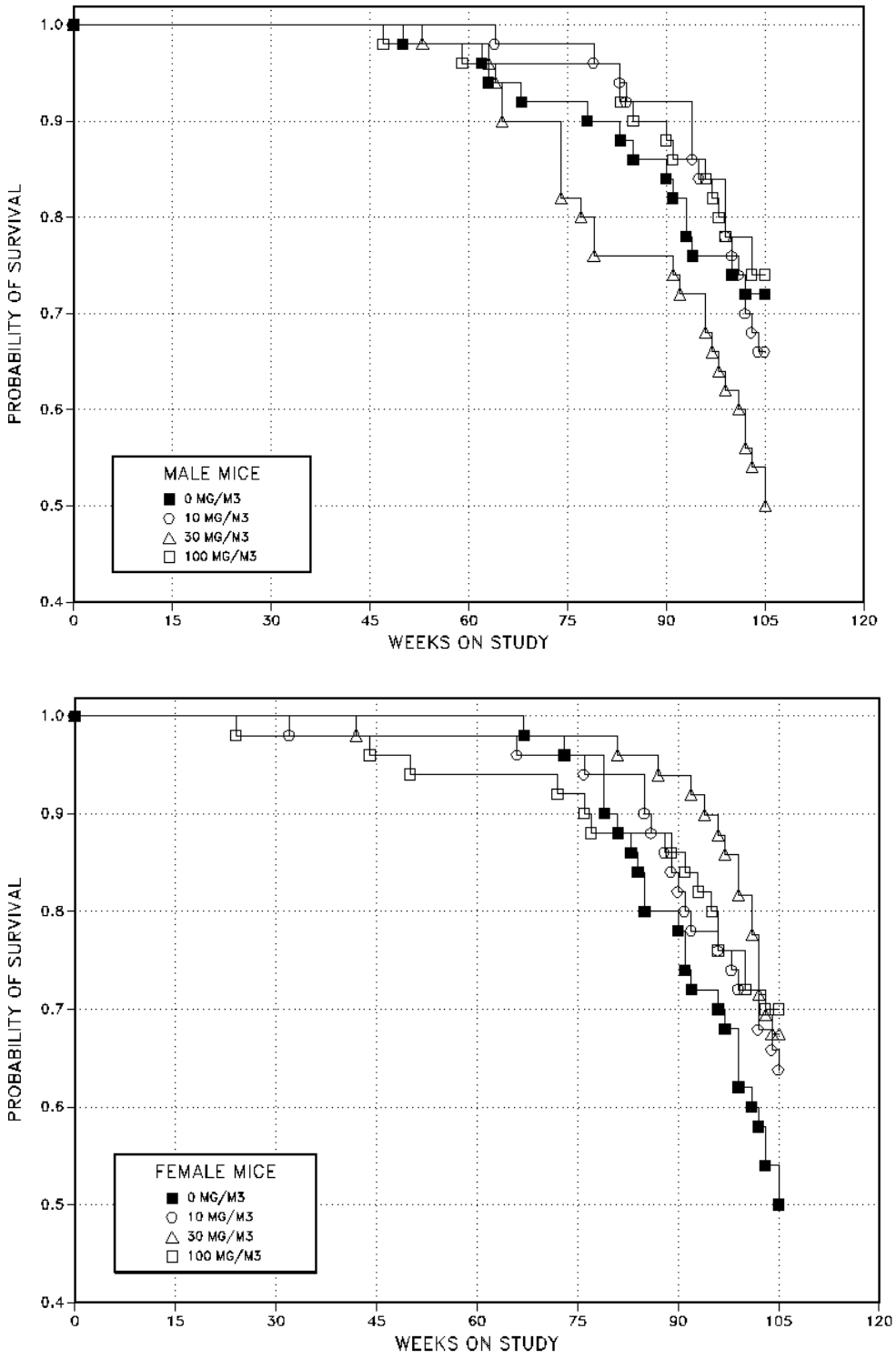
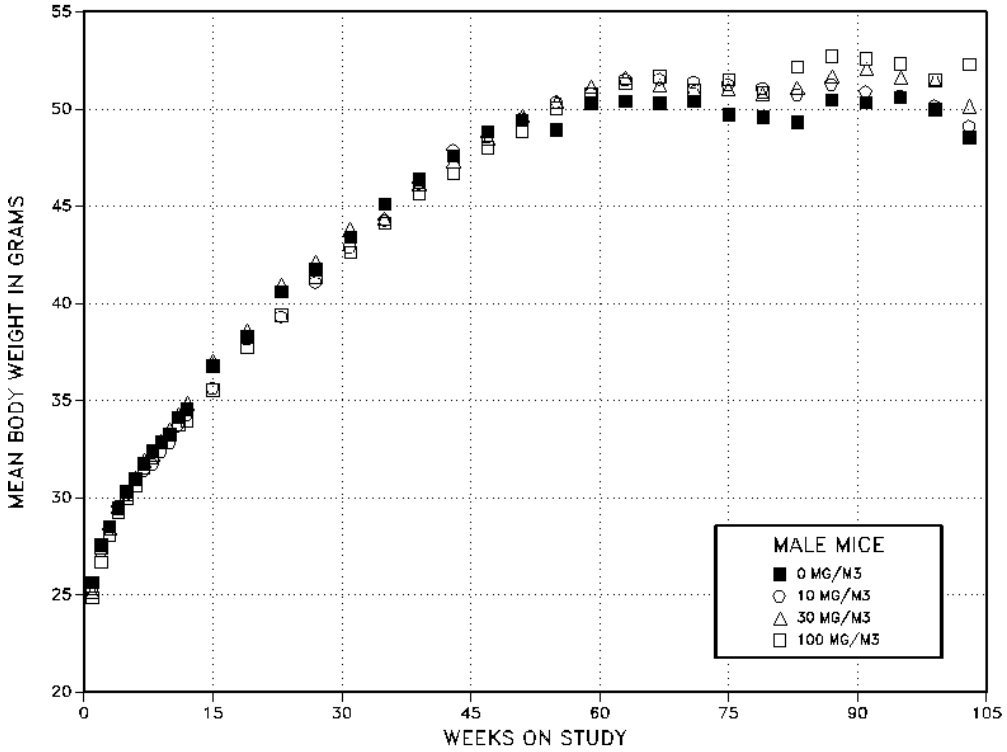


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered Molybdenum Trioxide by Inhalation for 2 Years



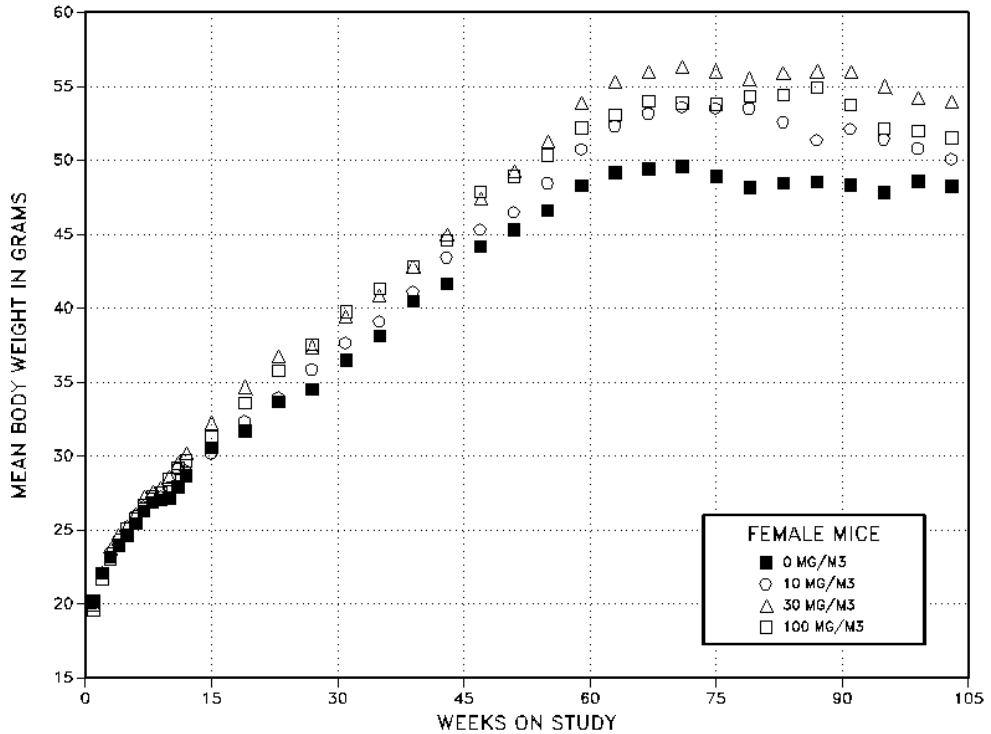


FIGURE 4
Growth Curves for Male and Female Mice
Administered Molybdenum Trioxide by Inhalation for 2 Years

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Molybdenum Trioxide

Weeks on Study	0 mg/m^3		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	25.6	50	25.3	99	50	25.1	98	50	24.8	97	50
2	27.6	50	27.3	99	50	27.5	100	50	26.7	97	50
3	28.5	50	28.4	100	50	28.4	100	50	28.0	98	50
4	29.5	50	29.5	100	50	29.5	100	50	29.2	99	50
5	30.3	50	30.2	100	50	30.2	100	50	29.9	99	50
6	31.0	50	31.0	100	50	31.0	100	50	30.6	99	50
7	31.7	50	31.4	99	50	31.9	101	50	31.5	99	50
8	32.4	50	31.7	98	50	32.2	99	50	32.0	99	50
9	32.9	50	32.3	98	50	32.9	100	50	32.9	100	50
10	33.3	50	32.8	99	50	33.5	101	50	33.3	100	50
11	34.1	50	33.7	99	50	34.3	101	50	33.7	99	50
12	34.5	50	34.2	99	50	34.8	101	50	33.9	98	50
15	36.8	50	35.6	97	50	37.0	101	50	35.5	97	50
19	38.3	50	38.2	100	50	38.6	101	50	37.8	99	50
23	40.6	50	39.3	97	50	41.0	101	50	39.4	97	50
27	41.7	50	41.1	99	50	42.1	101	50	41.3	99	50
31	43.4	50	42.9	99	50	43.8	101	50	42.6	98	50
35	45.1	50	44.3	98	50	44.4	98	50	44.1	98	50
39	46.4	50	46.2	100	50	46.1	99	50	45.6	98	50
43	47.6	50	47.9	101	50	47.3	99	50	46.7	98	50
47	48.8	50	48.5	99	50	48.5	99	50	48.0	98	50
51	49.4	49	49.5	100	50	49.6	100	50	48.9	99	49
55	48.9	49	50.4	103	50	50.4	103	49	50.1	103	49
59	50.3	49	50.8	101	50	51.2	102	49	50.8	101	48
63	50.4	47	51.5	102	50	51.6	102	48	51.3	102	48
67	50.3	47	51.5	102	49	51.2	102	45	51.7	103	48
71	50.4	46	51.4	102	49	51.0	101	45	51.0	101	48
75	49.7	46	51.3	103	49	51.0	103	41	51.5	104	48
79	49.6	45	51.1	103	48	50.8	102	39	50.9	103	48
83	49.3	45	50.7	103	48	51.1	104	38	52.1	106	46
87	50.4	43	51.3	102	46	51.7	103	38	52.7	105	45
91	50.3	42	50.9	101	46	52.1	104	37	52.6	105	44
95	50.6	38	50.7	100	42	51.6	102	36	52.3	103	43
99	50.0	38	50.2	100	41	51.5	103	32	51.5	103	39
103	48.5	36	49.1	101	35	50.1	103	27	52.3	108	38
Mean for weeks											
1-13	31.0		30.7	99		30.9	100		30.5	98	
14-52	43.8		43.4	99		43.8	100		43.0	98	
53-103	49.9		50.8	102		51.2	103		51.6	103	

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Molybdenum Trioxide

Weeks on Study	0 mg/m^3		10 mg/m^3			30 mg/m^3			100 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.2	50	20.0	99	50	19.9	99	50	19.5	97	50
2	22.1	50	22.1	100	50	22.1	100	50	21.7	98	50
3	23.1	50	23.4	101	50	23.7	103	50	23.0	100	50
4	23.9	50	24.3	102	50	24.6	103	50	24.0	100	50
5	24.6	50	25.2	102	50	25.3	103	50	25.1	102	50
6	25.4	50	26.0	102	50	26.0	102	50	25.8	102	50
7	26.3	50	26.6	101	50	27.3	104	50	26.7	102	50
8	26.8	50	27.1	101	50	27.5	103	50	27.3	102	50
9	27.0	50	27.2	101	50	27.8	103	50	27.5	102	50
10	27.2	50	27.9	103	50	28.6	105	50	28.4	104	50
11	27.9	50	29.1	104	50	29.5	106	50	29.2	105	50
12	28.7	50	29.0	101	50	30.2	105	50	29.7	104	50
15	30.5	50	30.2	99	50	32.2	106	50	31.3	103	50
19	31.7	50	32.4	102	50	34.7	110	50	33.6	106	50
23	33.7	50	34.0	101	50	36.7	109	50	35.8	106	50
27	34.5	50	35.8	104	50	37.4	108	50	37.5	109	49
31	36.5	50	37.7	103	50	39.5	108	50	39.7	109	49
35	38.1	50	39.1	103	49	40.9	107	50	41.3	108	49
39	40.5	50	41.1	102	49	42.8	106	50	42.8	106	49
43	41.6	50	43.4	104	49	44.9	108	49	44.6	107	49
47	44.2	50	45.3	103	49	47.4	107	49	47.9	108	48
51	45.3	50	46.5	103	49	49.2	109	49	48.9	108	47
55	46.6	50	48.4	104	49	51.3	110	49	50.3	108	47
59	48.3	50	50.7	105	49	53.9	112	49	52.2	108	47
63	49.2	50	52.3	106	49	55.3	112	49	53.1	108	47
67	49.4	50	53.2	108	48	56.0	113	49	54.0	109	47
71	49.6	49	53.6	108	48	56.3	114	49	53.9	109	47
75	48.9	48	53.5	109	48	56.0	115	49	53.8	110	46
79	48.2	46	53.5	111	47	55.5	115	49	54.3	113	44
83	48.5	44	52.6	109	47	55.9	115	47	54.4	112	44
87	48.5	40	51.4	106	44	56.0	116	46	54.9	113	44
91	48.3	38	52.1	108	40	55.9	116	46	53.8	111	42
95	47.8	36	51.4	108	39	55.0	115	44	52.1	109	41
99	48.6	34	50.8	105	37	54.2	112	41	52.0	107	38
103	48.2	29	50.1	104	33	54.0	112	35	51.5	107	35
Mean for weeks											
1-13	25.3		25.7	102		26.0	103		25.7	102	
14-52	37.7		38.6	102		40.6	108		40.3	107	
53-103	48.5		51.8	107		55.0	113		53.1	109	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the respiratory system (lung, nose, and larynx). Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Respiratory System: The incidences of alveolar/bronchiolar carcinoma were significantly greater in all exposed groups of males than in the control group and exceeded the historical control range for 2-year NTP inhalation studies (Tables 13 and C4). The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in male mice exposed to 10 or 30 mg/m^3 were also significantly greater than that in the control group (Tables 13 and C3) and exceeded the range of historical controls (Tables 13 and C4). Female mice exposed to 30 or 100 mg/m^3 had significantly greater incidences of alveolar/bronchiolar adenoma than did the control group and the incidences exceeded the range of historical controls (Tables 13 and D4). The incidence of carcinoma was greater in female mice exposed to 100 mg/m^3 than in the control group, although the increase was not significant (Tables 13 and D3). However, the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in female mice exposed to 100 mg/m^3 was significantly greater than that in the control group and exceeded the range of historical controls (Tables 13 and D4). The combined incidence increased with a significant positive trend.

Alveolar adenomas were discrete nodular masses composed of well-differentiated epithelial cells that formed papillary or solid patterns distorting the underlying architecture and sometimes compressing the adjacent alveolar parenchyma (Plates 9 and 10). Papillary neoplasms were composed of irregular papillary structures lined by uniform cuboidal cells with abundant cytoplasm and round to oval nuclei. Solid neoplasms were composed of sheets of uniform polygonal cells with abundant eosinophilic cytoplasm and round, oval, or polygonal nuclei. Carcinomas were expansive, and at times invasive, masses composed of a pleomorphic population of anaplastic cells in papillary or pleomorphic growth patterns (Plate 11). Mitotic figures varied in number but were often numerous.

Incidences of metaplasia of the alveolar epithelium of minimal severity in the centriacinar region of the lung were significantly increased in all exposed groups of males and females (Tables 13, C5, and D5). The average severity of metaplasia was graded as minimal. Metaplasia was characterized by a change from the flattened epithelial cell type normally lining the alveolar ducts and adjacent alveolar septa to low cuboidal cells considered to be either Clara and/or Type II pneumocytes (Plate 12). The incidences of histiocyte cellular infiltration in all exposed groups of males were significantly greater than that in the control group. The incidence correlated to the incidence of alveolar/bronchiolar carcinoma and might be related to the presence of carcinomas in the lung.

In the nose, incidences of hyaline degeneration of the respiratory epithelium in 100 mg/m^3 males and females and hyaline degeneration of the olfactory epithelium in 100 mg/m^3 females were significantly greater than those in the control groups (Tables 13, C5, and D5). The degree of severity was generally minimal in all exposed and control groups, and, as in rats, this was considered a nonspecific degenerative response to chronic inhalation of molybdenum trioxide. Hyaline degeneration generally affected the respiratory epithelium lining the nasal septum in levels I and II of the nasal cavity, the medial surfaces of the nasoturbinates in level II, and was confined to the olfactory epithelium lining the dorsal meatus of level II. Microscopically, the lesions at both sites were morphologically similar to those observed in rats. The incidences of minimal focal suppurative inflammation in the nasal cavity of 30 and 100 mg/m^3 males and of minimal to mild focal atrophy of the olfactory epithelium of 100 mg/m^3 males were slightly greater than those of the control group. However, the degree of severity was similar to that of the control group, and it was not clear whether these lesions were related to chemical exposure. In the larynx, the incidences of squamous metaplasia of the epithelium lining the base of the epiglottis were significantly increased in all exposed groups of males and females (Tables 13, C5, and D5). However, the degree of

TABLE 13
Incidences of Selected Respiratory System Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Inhalation Study of Molybdenum Trioxide

	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Nose[a]	50	50	49	50
Inflammation, Suppurative[b]	2 (1.0)[c]	6 (1.0)	10* (1.2)	8* (1.1)
Olfactory Epithelium, Atrophy	3 (1.0)	5 (1.8)	3 (2.0)	10* (1.8)
Respiratory Epithelium, Degeneration, Hyaline	11 (1.2)	13 (1.0)	11 (1.0)	41** (1.1)
Larynx	50	49	48	50
Hyperplasia	1 (1.0)	3 (1.0)	6 (1.0)	41** (1.5)
Epiglottis, Metaplasia, Squamous	0	26** (1.0)	37** (1.3)	49** (2.2)
Lung	50	50	49	50
Infiltration Cellular, Histiocyte	2 (2.5)	16** (2.8)	9* (2.4)	9* (2.3)
Alveolar Epithelium, Metaplasia	0	32** (1.0)	36** (1.0)	49** (1.1)
Alveolar/bronchiolar Adenoma				
Overall rate[d]	9/50 (18%)	14/50 (28%)	10/49 (20%)	9/50 (18%)
Adjusted rate[e]	23.5%	37.8%	29.8%	22.5%
Terminal rate[f]	7/36 (19%)	11/33 (33%)	5/25 (20%)	7/37 (19%)
First incidence (days)	651	653	440	576
Logistic regression test[g]	P=0.322N	P=0.205	P=0.464	P=0.571N
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	2/50 (4%)	16/50 (32%)	14/49 (29%)	10/50 (20%)
Adjusted rate	4.9%	40.7%	43.3%	25.3%
Terminal rate	1/36 (3%)	11/33 (33%)	8/25 (32%)	8/37 (22%)
First incidence (days)	544	580	513	629
Logistic regression test	P=0.385	P<0.001	P<0.001	P=0.017
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	11/50 (22%)	27/50 (54%)	21/49 (43%)	18/50 (36%)
Adjusted rate	27.7%	64.8%	56.1%	43.4%
Terminal rate	8/36 (22%)	19/33 (58%)	10/25 (40%)	14/37 (38%)
First incidence (days)	544	580	440	576
Logistic regression test	P=0.541N	P=0.001	P=0.020	P=0.106
Female				
Nose	49	50	49	49
Olfactory Epithelium, Degeneration, Hyaline	22 (1.4)	14 (1.1)	14 (1.2)	36** (1.5)
Respiratory Epithelium, Degeneration, Hyaline	26 (1.2)	23 (1.0)	28 (1.2)	48** (1.8)
Larynx	49	50	49	50
Hyperplasia	1 (2.0)	1 (1.0)	7 (1.3)	35** (1.9)
Epiglottis, Metaplasia, Squamous	1 (1.0)	36** (1.1)	43** (1.5)	49** (2.2)

(continued)

TABLE 13
Incidences of Selected Respiratory System Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Inhalation Study of Molybdenum Trioxide (continued)

	0 mg/m^3	10 mg/m^3	30 mg/m^3	100 mg/m^3
Female (continued)				
Lung	50	50	49	49
Alveolar Epithelium, Metaplasia	2 (1.0)	26** (1.1)	39** (1.1)	46** (1.1)
Alveolar/bronchiolar Adenoma[i]				
Overall rate	1/50 (2%)	4/50 (8%)	8/49 (16%)	9/49 (18%)
Adjusted rate	3.7%	11.0%	23.5%	24.8%
Terminal rate	0/25 (0%)	2/31 (6%)	7/33 (21%)	8/35 (23%)
First incidence (days)	729	610	720	667
Logistic regression test	P=0.018	P=0.184	P=0.036	P=0.016
Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	0/49 (0%)	6/49 (12%)
Adjusted rate	5.8%	5.2%	0.0%	16.3%
Terminal rate	0/25 (0%)	0/31 (0%)	0/33 (0%)	5/35 (14%)
First incidence (days)	642	629	—[k]	632
Logistic regression test	P=0.024	P=0.694	P=0.256N	P=0.140
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	3/50 (6%)	6/50 (12%)	8/49 (16%)	15/49 (31%)
Adjusted rate	9.3%	15.6%	23.5%	40.1%
Terminal rate	0/25 (0%)	2/31 (6%)	7/33 (21%)	13/35 (37%)
First incidence (days)	642	610	720	632
Logistic regression test	P<0.001	P=0.223	P=0.152	P=0.003

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
a Number of animals with organ examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
d Number of animals with neoplasm per number of animals with organ examined microscopically
e Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
f Observed incidence in animals surviving until the end of the study
g In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. For all tests, a negative trend or a lower incidence in an exposure group is indicated by **N**.
h Historical incidence for 2-year NTP inhalation studies with untreated controls (mean ± standard deviation): 75/947 (7.9% ± 5.7%); range 0%-16%
i Historical incidence: 205/947 (21.7% ± 8.0%); range 10%-42%
j Historical incidence: 61/939 (6.5% ± 3.2%); range 0%-14%
k Not applicable; no neoplasms in animal group
l Historical incidence: 97/939 (10.3% ± 3.7%); range 0%-16%

severity was generally minimal in the 10 and 30 mg/m^3 groups and mild in the 100 mg/m^3 groups. Squamous metaplasia was similar to that observed in rats. The normally single layer of pseudostratified ciliated cuboidal to low columnar epithelium lining the base of the epiglottis was replaced by an epithelium that consisted of a hypercellular layer of basal cells with one or more superficial layers of flattened (squamous) epithelial cells that lacked cilia. In advanced cases, there was slight keratinization of the epithelial surface. In both male and female mice, the incidences of hyperplasia in level II of the laryngeal epithelium increased with increasing exposure concentration. The increase was statistically significant only in mice exposed to 100 mg/m^3 with 82% of male and 70% of female mice affected. This lesion was characterized by increased thickness of the stratified squamous epithelium that normally covers the medial and ventral aspects of the corniculate processes of the arytenoid cartilage of the vocal cords. Because the severity of both squamous metaplasia of the epiglottis and hyperplasia of the laryngeal epithelium did not vary significantly between exposed and control groups, these lesions were not considered to represent adaptive responses to chronic inhalation of molybdenum trioxide.

GENETIC TOXICOLOGY

Molybdenum trioxide (10-10,000 µg/plate) did not induce mutations in *Salmonella typhimurium* strain TA97, TA98, TA100, TA1535, or TA1537, with or without induced hamster or rat liver S9 (Table E1). No induction of sister chromatid exchanges (Table E2) or chromosomal aberrations (Table E3) was observed, with or without S9, in cytogenetic tests with cultured Chinese hamster ovary cells.

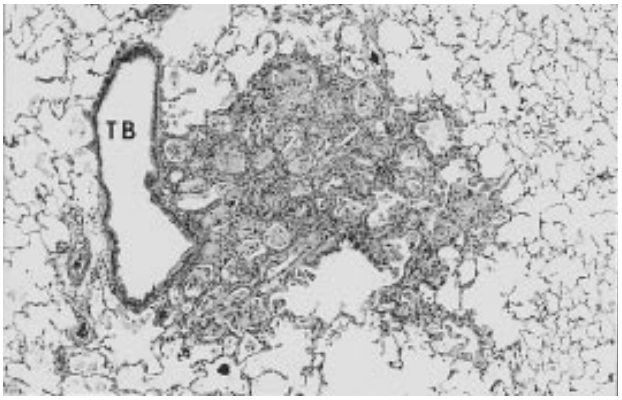


PLATE 1
Peribronchiolar chronic inflammation in the lung of a female rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. TB=terminal bronchiole. H&E; 45×

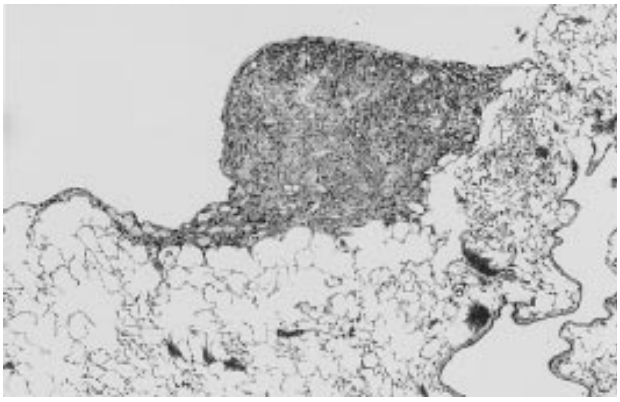


PLATE 2
Subpleural chronic inflammation in the lung of a male rat exposed to 30 mg/m^3 molybdenum trioxide by inhalation for 2 years. H&E; 36×

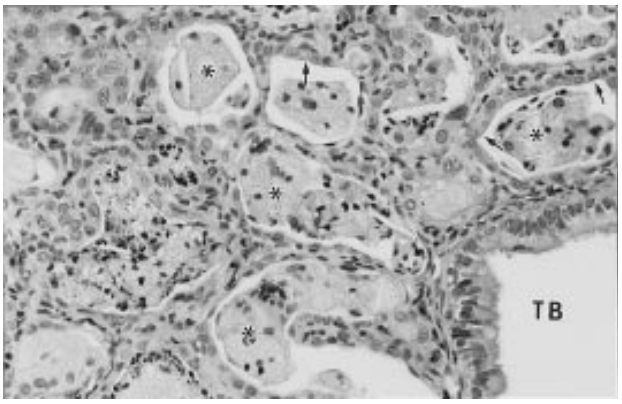


PLATE 3
Peribronchiolar chronic inflammation in the lung of a male rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Alveoli contain aggregates of large foamy macrophages (asterisks). Alveolar septae are lined by cuboidal hyperplastic type II cells (arrows). TB=terminal bronchiole. H&E; 180×

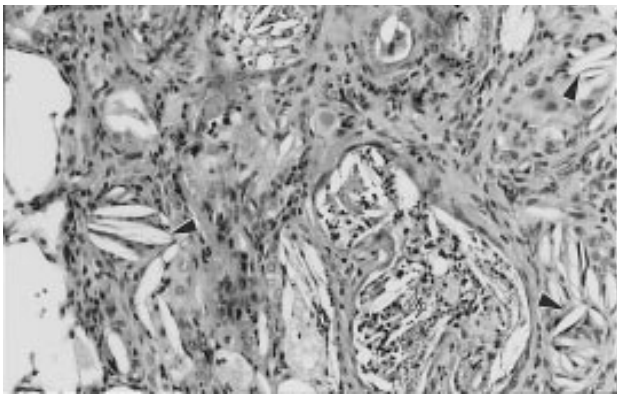


PLATE 4
Chronic inflammation in the lung of a female rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Note macrophages within alveoli and alveolar septae, type II cell hyperplasia, and cholesterol clefts (arrowheads). An alveolus contains aggregates of neutrophils mixed with cellular debris (asterisk). H&E; 150×

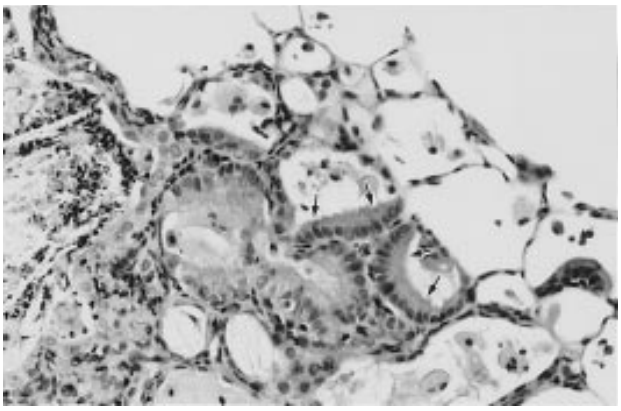


PLATE 5
Alveolar epithelial metaplasia in a focus of chronic inflammation in the lung of a female rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Alveolar septae are lined by ciliated columnar epithelial cells (arrows). Note macrophages within alveoli. H&E; 180×

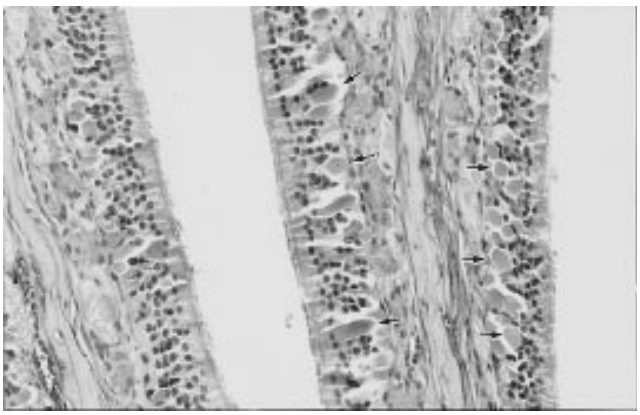


PLATE 6
Hyaline degeneration of the olfactory epithelium in level III of the nasal cavity of a male rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Large eosinophilic globules (arrows) fill the cytoplasm of epithelial cells of the ethmoid turbinates. The epithelium has fewer cell layers and there is disorganization of the normally layered rows of sensory cell nuclei. H&E; 180×

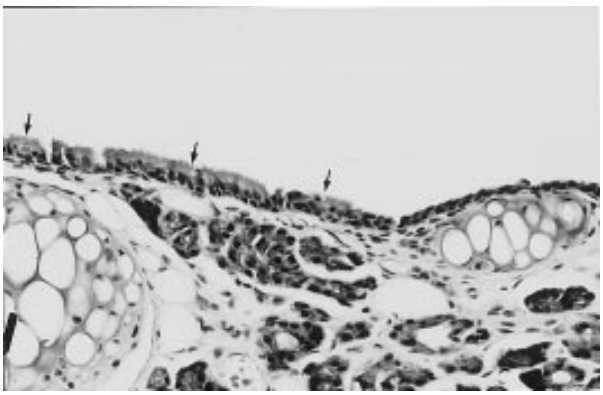


PLATE 7
Normal ciliated columnar epithelium lining the base of the epiglottis (arrows) of a male rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. H&E; 180×

PLATE 8
Squamous metaplasia of the epithelium at the base of the epiglottis of a male rat exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Flattened (squamous), non-ciliated epithelium (arrows) replaces the ciliated columnar epithelium normally present at this location. H&E; 56×

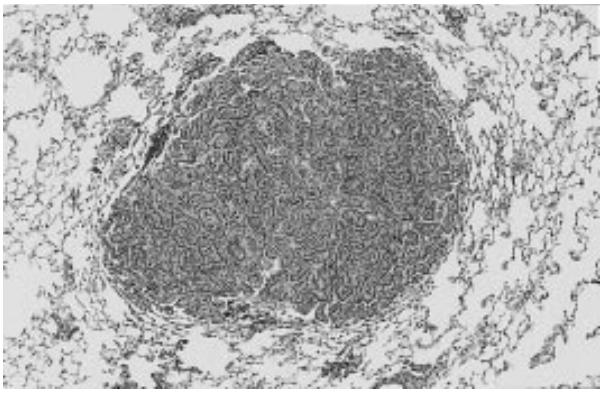


PLATE 9
Alveolar/bronchiolar adenoma in the lung of a male mouse exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. H&E; 45×

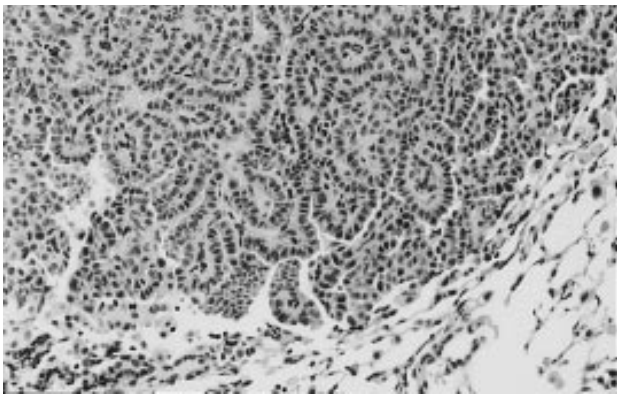


PLATE 10
Higher magnification of Plate 9. The alveolar architecture is effaced by uniform, well-differentiated cuboidal neoplastic epithelial cells arranged in a papillary pattern. H&E; 150×

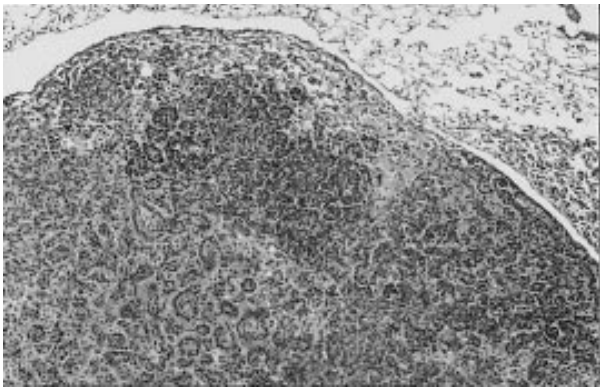


PLATE 11
Alveolar/bronchiolar carcinoma in the lung of a male mouse exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Anaplastic epithelial cells in pleomorphic growth patterns efface the alveolar architecture. H&E; 60×

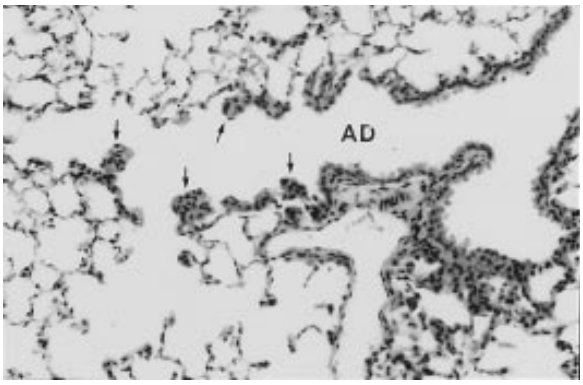


PLATE 12
Epithelial metaplasia in the centriacinar region of the lung of a female mouse exposed to 100 mg/m^3 molybdenum trioxide by inhalation for 2 years. Cuboidal (metaplastic) epithelial cells (arrows) replace the normally flattened epithelial cells lining the alveolar duct (AD) and the adjacent alveolar septae. H&E; 125×

DISCUSSION AND CONCLUSIONS

Molybdenum trioxide is water soluble and is well absorbed after inhalation or oral exposure. Fairhall *et al.* (1945) reported that in guinea pigs exposed to molybdenum trioxide at 204.7 mg/m^3 for 1 hour per day, 5 days per week, for 5 weeks, molybdenum concentrations in liver, kidney, lung, spleen, and bone were higher than those of the controls. In the present studies, blood molybdenum concentrations in rats and mice increased proportionally with increasing exposure concentration; male rats exhibited higher blood molybdenum concentrations and greater variability than female rats. Blood molybdenum concentrations of male rats were higher than those of mice exposed to the same concentration. This was probably due to the larger lung capacity of rats and, therefore, more molybdenum trioxide was inhaled. The blood levels of molybdenum in rats and mice in these studies demonstrated that molybdenum trioxide administered via inhalation was well absorbed.

Exposure of rats and mice to molybdenum trioxide for 14 days at concentrations of 0, 3, 10, 30, 100, or 300 mg/m^3 had no effect on survival or clinical findings. However, final mean body weights of male and female rats and mice exposed to 300 mg/m^3 were significantly lower than those of the control groups. No chemical-related lesions were observed in rats or mice. Because of the body weight effects in the 14-day studies, rats and mice were exposed to 0, 1, 3, 10, 30, or 100 mg/m^3 molybdenum trioxide during the 13-week studies. There were no effects on survival, final mean body weights, clinical findings, organ weights, clinical pathology parameters, sperm counts, or sperm motility. The concentrations of copper in the liver of male and female mice exposed to 100 mg/m^3 were significantly greater than those of the control groups. The significance of the increased liver copper concentrations in mice is not known. The concentrations of copper in the liver of exposed male and female rats were similar to those of the control groups. No chemical-related lesions were observed in rats or mice.

Exposure of rats to molybdenum trioxide for 2 years at concentrations of 0, 10, 30, or 100 mg/m^3 had no effect on survival, body weight gain, clinical findings, or bone density or curvature. Exposure to molybdenum is known to cause deformities in the joints (USEPA, 1975). In the present studies, no bone or joint abnormalities were observed.

The rats did not show any typical symptoms of molybdenum toxicity such as diarrhea, alopecia, achromotrichia, dermatosis, or anemia. The amount of molybdenum absorbed in the present studies was probably below the threshold level needed to initiate such symptoms of toxicity. The bone and joint effect of molybdenum may be more difficult to manifest in young adult rats (7-8 weeks old) when the skeletal system is almost fully developed. The bone and joint effects were demonstrated by Miller *et al.* (1956), Van Reen (1959), and Lalich *et al.* (1965) when weanling rats were exposed to high molybdenum and low copper concentrations. The concentration of copper in the diet (4 mg/kg) might have protected the rats from molybdenum toxicity to a certain extent.

Exposure of male and female rats to molybdenum trioxide resulted in the development of respiratory system lesions. In the lung, the incidence and severity of chronic alveolar inflammation increased with increasing exposure concentration in male and female rats. In addition, four alveolar/bronchiolar adenomas or carcinomas were present in male rats exposed to 100 mg/m^3; none were present in controls. However, the combined incidence of these neoplasms was within the range of historical controls for 2-year NTP inhalation studies. No carcinogenic response was observed in female rats exposed to molybdenum trioxide. The lesions in the nose (hyaline degeneration) and larynx (squamous metaplasia) were considered to be nonspecific defensive or adaptive responses to the chronic inhalation exposure to molybdenum trioxide.

Bompart *et al.* (1990) reported that molybdenum salt administered at 80 mg/kg orally for 8 weeks induced mild renal failure in male Sprague-Dawley rats. USEPA (1975) reported fatty degeneration in liver and kidney. In the present studies, F344/N rats exposed to

molybdenum trioxide at concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 for 13 weeks or at concentrations of 0, 10, 30, or 100 mg/m^3 for 2 years did not show any signs or symptoms of renal effects. Kidney weights and creatinine concentrations of exposed rats in the 13-week study were similar to those of the controls. No liver lesions were observed.

High intake of molybdenum significantly increased serum and tissue molybdenum concentrations in sheep (Pitt *et al.*, 1980) and in rats (Seaborn and Yang, 1993). In female Sprague-Dawley rats, higher intake of molybdenum resulted in proportionally higher excretion in urine and feces (Seaborn and Yang, 1993). Renal and urinary copper concentrations were increased in sheep following molybdenum ingestion (Marcilese *et al.*, 1969). However, Seaborn and Yang (1993) did not notice any increase in copper excretion in female Sprague-Dawley rats exposed to molybdenum in drinking water. There were no significant differences in the concentration of copper in the liver of exposed and control rats in the current 13-week study.

Exposure of mice to molybdenum trioxide for 2 years at concentrations of 0, 10, 30, or 100 mg/m^3 had no effect on survival, clinical findings, or bone density or curvature. Final mean body weights of 30 mg/m^3 and 100 mg/m^3 females were greater than that of the control group.

In contrast to rats, exposure of mice to molybdenum trioxide was associated with the development of lung neoplasms. The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in male mice exceeded the historical control range for 2-year NTP inhalation studies. In female mice, the incidences of alveolar/bronchiolar adenoma in the 30 and 100 mg/m^3 groups and of alveolar/bronchiolar adenoma or carcinoma (combined) in the 100 mg/m^3 group were significantly greater than those in the control group. Unlike in rats, chronic inflammatory lesions were not present in the lungs of mice. The nose and larynx lesions present in mice were similar to those observed in rats and were considered to be nonspecific, defensive, or adaptive responses to chronic inhalation exposure to molybdenum trioxide.

Inherent species differences may explain the pulmonary responses associated with molybdenum trioxide exposure in rats and mice. Fischer 344/N rats are known to have a low incidence of spontaneous lung neoplasms, whereas the spontaneous incidence of lung neoplasms in B6C3F$_1$ mice is relatively high. In NTP studies, chemical-associated increased incidences of lung neoplasms occur twice as often in mice as in rats. Rats are known to develop chronic inflammatory lesions in the lung following inhalation exposure to particulates, and in many of these studies increased incidences of lung neoplasms also occur. The distribution of chronic inflammatory lesions observed in the lungs of rats in this study is similar to that observed in other inhalation studies of particulate compounds, with lesions developing adjacent to terminal bronchioles and alveolar ducts (centriacinar region of the lung) and in subpleural sites (Lee, 1989). Qualitatively, these lesions are similar to those observed in other inhalation studies of particulates. However, the severity of chronic inflammation in rats in the present study is rather mild compared to that observed in other particulate studies with talc, nickel oxide, and nickel subsulfide (NTP, 1993; NTP, 1996a,b).

Molybdenum trioxide is not mutagenic. The mechanism of action of molybdenum trioxide in lung carcinogenesis is not known. The nonneoplastic lesions observed in the nose and larynx of rats and in the nose, larynx, and lung of mice were apparently attempts by the rats and mice to develop a more durable epithelium in response to the chronic effects of molybdenum trioxide exposure. Pneumoconiosis has been described by Friberg (1979) in experimental animals exposed to molybdenum trioxide subchronically.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity** of molybdenum trioxide in male F344/N rats based on a marginally significant positive trend of alveolar/bronchiolar adenoma or carcinoma (combined). There was *no evidence of carcinogenic activity* of molybdenum trioxide in female F344/N rats exposed to 10, 30, or 100 mg/m^3. There was *some evidence of carcinogenic activity* of molybdenum trioxide in male B6C3F$_1$ mice based on increased

incidences of alveolar/bronchiolar carcinoma and adenoma or carcinoma (combined). There was *some evidence of carcinogenic activity* of molybdenum trioxide in female B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined).

Exposure of male and female rats to molybdenum trioxide by inhalation resulted in increased incidences of chronic alveolar inflammation, hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), and squamous metaplasia of the epiglottis.

Exposure of male and female mice to molybdenum trioxide by inhalation resulted in increased incidences of metaplasia of the alveolar epithelium, histiocytic cellular infiltration (males), hyaline degeneration of the respiratory epithelium, hyaline degeneration of the olfactory epithelium (females), squamous metaplasia of the epiglottis, and hyperplasia of the larynx.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

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Copper

Fact Sheet for Health Professionals

Consumer Datos en español
Health Professional
Other Resources

This is a fact sheet intended for health professionals. For a general overview, see our consumer fact sheet.

Introduction

Copper, an essential mineral, is naturally present in some foods and is available as a dietary supplement. It is a cofactor for several enzymes (known as cuproenzymes) involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis [1-3]. One abundant cuproenzyme is ceruloplasmin (CP), which plays a role in iron metabolism and carries more than 95% of the total copper in healthy human plasma [4]. Copper is also involved in many physiologic processes, such as angiogenesis; neurohormone homeostasis; and regulation of gene expression, brain development, pigmentation, and immune system functioning [1]. In addition, defense against oxidative damage depends mainly on the copper-containing superoxide dismutases [5,6].

A wide variety of plant and animal foods contain copper, and the average human diet provides approximately 1,400 mcg/day for men and 1,100 mcg/day for women that is primarily absorbed in the upper small intestine [1,2,7-9]. Almost two-thirds of the body's copper is located in the skeleton and muscle [1,3].

Only small amounts of copper are typically stored in the body, and the average adult has a total body content of 50–120 mg copper [1,2]. Most copper is excreted in bile, and a small amount is excreted in urine. Total fecal losses of copper of

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biliary origin and nonabsorbed dietary copper are about 1 mg/day [1,2]. Copper levels in the body are homeostatically maintained by copper absorption from the intestine and copper release by the liver into bile to provide protection from copper deficiency and toxicity [3].

Copper status is not routinely assessed in clinical practice, and no biomarkers that accurately and reliably assess copper status have been identified [2]. Human studies typically measure copper and cuproenzyme activity in plasma and blood cells because individuals with known copper deficiency often have low blood levels of copper and CP [2]. However, plasma CP and copper levels can be influenced by other factors, such as estrogen status, pregnancy, infection, inflammation, and some cancers [2]. Normal serum concentrations are 10–25 mcmol/L (63.5–158.9 mcg/dL) for copper and 180–400 mg/L for CP [10].

Recommended Intakes

Intake recommendations for copper and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the National Academies of Sciences, Engineering, and Medicine [3]. DRI is the general term for a set of reference values used for planning and assessing nutrient intakes of healthy people. These values, which vary by age and sex, include the following:

- Recommended Dietary Allowance (RDA): Average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals; often used to plan nutritionally adequate diets for individuals
- Adequate Intake (AI): Intake at this level is assumed to ensure nutritional adequacy; established when evidence is insufficient to develop an RDA
- Estimated Average Requirement (EAR): Average daily level of intake estimated to meet the requirements of 50% of healthy individuals; usually used to assess the nutrient intakes of groups of people and to plan nutritionally adequate diets for them; can also be used to assess the nutrient intakes of individuals
- Tolerable Upper Intake Level (UL): Maximum daily intake unlikely to cause adverse health effects

Table 1 lists the current RDAs for copper [3]. For infants from birth to 12 months, the FNB established an AI for copper that is equivalent to the mean intake of copper in healthy, breastfed infants.

Table 1: Recommended Dietary Allowances (RDAs) for Copper [3]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months*	200 mcg	200 mcg		
7–12 months*	220 mcg	220 mcg		
1–3 years	340 mcg	340 mcg		
4–8 years	440 mcg	440 mcg		
9–13 years	700 mcg	700 mcg		
14–18 years	890 mcg	890 mcg	1,000 mcg	1,300 mcg
19+ years	900 mcg	900 mcg	1,000 mcg	1,300 mcg

*Adequate Intake (AI)

Sources of Copper

Food

The richest dietary copper sources include shellfish, seeds and nuts, organ meats, wheat-bran cereals, whole-grain products, and chocolate [1,2]. The absorption of copper is strongly influenced by the amount of copper in the diet; bioavailability ranges from 75% of dietary copper when the diet contains only 400 mcg/day to 12% when the diet contains 7.5 mg/day [3].

Tap water and other beverages can also be sources of copper, although the amount of copper in these liquids varies by source (ranging from 0.0005 mg/L to 1 mg/L) [2,11].

Several food sources of copper are listed in Table 2.

Table 2: Copper Content of Selected Foods [12]

Food	Micrograms (mcg) per serving	Percent DV*
Beef, liver, pan fried (3 ounces)	12,400	1,378
Oysters, eastern, wild, cooked, 3 ounces	4,850	539
Baking chocolate, unsweetened, 1 ounce	938	104
Potatoes, cooked, flesh and skin, 1 medium potato	675	75

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Food	Micrograms (mcg) per serving	Percent DV*
Mushrooms, shiitake, cooked, cut pieces, ½ cup	650	72
Cashew nuts, dry roasted, 1 ounce	629	70
Crab, Dungeness, cooked, 3 ounces	624	69
Sunflower seed kernels, toasted, ¼ cup	615	68
Turkey, giblets, simmered, 3 ounces	588	65
Chocolate, dark, 70%–85% cacao solids, 1 ounce	501	56
Tofu, raw, firm, ½ cup	476	53
Chickpeas, mature sees, ½ cup	289	32
Millet, cooked, 1 cup	280	31
Salmon, Atlantic, wild, cooked, 3 ounces	273	30
Pasta, whole wheat, cooked, 1 cup (not packed)	263	29
Avocado, raw, ½ cup	219	24
Figs, dried, ½ cup	214	24
Spinach, boiled, drained, ½ cup	157	17
Asparagus, cooked, drained, ½ cup	149	17
Sesame seeds, ¼ cup	147	16
Turkey, ground, cooked, 3 ounces	128	14
Cereal, Cream of Wheat, cooked with water, stove top, 1 cup	104	12
Tomatoes, raw, chopped, ½ cup	53	6
Yogurt, Greek, plain, low fat, 7-ounce container	42	5
Milk, nonfat, 1 cup	27	3
Apples, raw, with skin, ½ cup slices	17	2

*DV = Daily Value. The U.S. Food and Drug Administration (FDA) developed DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. The DV for copper is 0.9 mg (900 mcg) for adults and children age 4 years and older [13]. FDA does not require food labels to list copper content unless copper has been added to the food. Foods providing 20% or more of the DV are considered to be high sources of a nutrient, but foods providing lower percentages of the DV also contribute to a healthful diet.

The U.S. Department of Agriculture's (USDA's) FoodData Central (https://fdc.nal.usda.gov/) [12] lists the nutrient content of many foods.

Dietary supplements

Copper is available in dietary supplements containing only copper, in supplements containing copper in combination with other ingredients, and in many multivitamin/mineral products [14]. These supplements contain many different forms of copper, including cupric oxide, cupric sulfate, copper amino acid chelates, and copper gluconate. To date, no studies have compared the bioavailability of copper from these and other forms [15]. The amount of copper in dietary supplements typically ranges from a few micrograms to 15 mg (about 17 times the DV for copper) [14].

Copper Intakes and Status

Typical diets in the United States meet or exceed the copper RDA. Mean dietary intakes of copper from foods range from 800 to 1,000 mcg per day for children age 2–19 [9]. In adults age 20 and older, average daily intakes of copper from food are 1,400 mcg for men and 1,100 mcg for women. Total intakes from supplements and foods are 900 to 1,100 mcg/day for children and 1,400 to 1,700 mcg/day for adults age 20 and over.

According to an analysis of data from the 2009–2012 National Health and Nutrition Survey (NHANES), 6% to 15% of adults age 19 and older who do not take dietary supplements containing copper have copper intakes below the EAR [16]. In those who do use supplements, rates of adults with intakes below the copper EAR range from 2.2% to 7.2%.

Copper Deficiency

Copper deficiency is uncommon in humans [2]. Based on studies in animals and humans, the effects of copper deficiency include anemia, hypopigmentation, hypercholesterolemia, connective tissue disorders, osteoporosis and other bone defects, abnormal lipid metabolism, ataxia, and increased risk of infection [1,17,18].

Groups at Risk of Copper Inadequacy

The following groups are most likely to have inadequate copper status.

People with celiac disease

In a study of 200 adults and children with celiac disease, of which 69.9% claimed to maintain a gluten-free diet, 15% had copper deficiency (less than 70 mcg/dL in serum in men and girls younger than 12 years and less than 80 mcg/dL in women older than 12 years and/or CP less than 170 mg/L) as a result of intestinal malabsorption resulting from the intestinal lining alterations associated with celiac disease [19]. In its 2009 clinical guidelines for celiac disease, the American College of Gastroenterology notes that people with celiac disease appear to have an increased risk of copper deficiency and that copper levels normalize within a month of adequate copper supplementation while eating a gluten-free diet [20].

People with Menkes disease

Menkes disease is a rare, X-linked, recessive disorder of copper homeostasis caused by *ATP7A* mutations, which encode a copper-transporting ATPase [1]. In these individuals, intestinal absorption of dietary copper drops sharply, leading to signs of copper deficiency, including low serum copper and CP levels [1,21]. The typical manifestations of Menkes disease include failure to thrive, impaired cognitive development, aortic aneurysms, seizures, and unusually kinky hair [22]. Most individuals with Menkes disease die by age 3 years if untreated, but subcutaneous injections of copper starting in the first few weeks after birth can reduce mortality risk and improve development [23].

People taking high doses of zinc supplements

High dietary intakes of zinc can interfere with copper absorption, and excessive use of zinc supplements can lead to copper deficiency. Reductions in erythrocyte copper-zinc superoxide dismutase, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks [3]. People who regularly consume high doses of zinc from supplements or use excessive amounts of zinc-containing denture creams can develop copper deficiency because zinc can inhibit copper absorption. This is one reason the FNB established the UL for zinc at 40 mg/day for adults [1,3].

Copper and Health

This section focuses on two diseases in which copper might play a role: cardiovascular disease (CVD) and Alzheimer's disease.

Cardiovascular disease

Copper deficiency leads to changes in blood lipid levels, a risk factor for atherosclerotic CVD [1]. Animal studies have shown that copper deficiency is associated with cardiac abnormalities, possibly because of the resulting decreases in the activity of several cardiac cuproenzymes [1,2].

However, observational studies of the link between copper concentrations and CVD have had mixed results. A representative cohort study of 1,197 asymptomatic adults age 45 to 64 in Italy assessed the effects of self-reported copper intakes on various metabolic markers, including markers of atherosclerotic disease risk (diastolic blood pressure, total and low-density lipoprotein [LDL] levels) [24]. Diastolic blood pressure, total cholesterol, and LDL cholesterol levels were significantly lower in the highest tertile of copper intake (2.29 mg/day) compared with the lowest tertile (1.12 mg/day). In contrast, an analysis of 1976–1992 data on 4,574 participants in the second NHANES found that the risk of death from coronary heart disease was 2.87 times higher for participants age 30 and older in the fourth quartile for serum copper concentration (137 mcg/dL or higher) than for those in the first quartile (less than 106 mcg/dL) [25]. Similarly, an analysis of data on 3,253 adults with acute coronary syndromes (mean age 62 years in the 70% who were male and 65 years in the 30% who were female) in a cardiovascular health study in Germany found higher hazard ratios—2.58 for copper and 3.02 for CP concentrations in serum—for death from CVD in the highest (mean 147 mcg/dL for copper, 38.3 mg/dL for CP) versus the lowest (81.6 mcg/dL for copper, 22.9 mg/dL for CP) quartiles [26].

A few small studies that assessed the impact of copper supplementation in healthy adults have found little evidence that supplementation affects CVD risk factors. For example, daily supplementation with 2 mg copper as copper glycinate for 8 weeks in 70 healthy adults age 45 to 60 years increased the activity of two cuproenzymes, erythrocyte superoxide dismutase 1 and plasma CP, but had no effect on five other CVD-related plasma markers (CRP, homocysteine, total cholesterol, high-density lipoprotein cholesterol, and LDL cholesterol) [27]. In 16 healthy women (mean age 24 years), daily supplementation with 3 mg or 6 mg elemental copper as copper

sulfate had no significant effect on CVD risk factors, including total plasma cholesterol or triacylglycerol concentrations [28]. However, the concentration of fibrinolytic factor PAI-I decreased by about 30% (indicating reduced CVD risk) with 6 mg/day copper supplementation compared with placebo. No clinical trials of copper supplementation have been conducted in people with increased CVD risk.

Overall, the evidence to date is insufficient to support any conclusions about the association between copper concentrations and CVD risk or the impact of copper supplementation on CVD.

Alzheimer's disease

Some experts believe that dietary copper deficiency plays a role in the etiology and pathophysiology of Alzheimer's disease, the leading cause of dementia, because of several reports of low copper levels and low activity of copper-dependent enzymes in the brains of people with the disease [7,29]. Limited evidence shows that people with higher copper levels have a lower risk of Alzheimer's disease [30]. However, high levels of copper have also been found in the brains of people with Alzheimer's disease, and some researchers argue that excess amounts of dietary copper are involved in the development of this disease [31]. Furthermore, copper accumulation in damaged brain regions in Alzheimer's disease might not directly reflect overall body copper status or copper intakes [32].

A few observational studies have assessed the relationship between dietary copper levels and Alzheimer's disease, with mixed results. One study, for example, assessed cognitive function using four cognitive tests during home visits every 3 years for 6 years and intakes of copper and saturated and trans fats using a food frequency questionnaire in 3,718 community-dwelling (noninstitutionalized) adults age 65 and older [33]. In the overall study population, dietary and total copper intakes were not associated with cognitive decline. However, in 604 participants (16.2%) who consumed a diet higher in saturated and trans fat, total copper intake in the highest quintile (median 2.75 mg/day) was associated with a significantly faster rate of cognitive decline compared with the lowest intake quintile (median 0.88 mg/day). In contrast, an analysis of data on 1,112 adults older than 60 years found no differences in serum copper or CP levels between patients with Alzheimer's disease (n=211) and healthy controls (n=695) [32]. This study did reveal, however, a significant decline in serum copper not bound to CP in patients

with mild cognitive impairment or Alzheimer's disease compared with the healthy control group 18 months after baseline.

Meta-analyses have found that people with Alzheimer's disease tend to have higher serum copper levels than adults without the disease. In a meta-analysis of 10 studies in 867 healthy individuals and 599 with Alzheimer's disease (mean age greater than 70 years in both groups), patients with Alzheimer's disease had significantly higher serum levels of copper not bound to CP and total serum copper than healthy controls [34]. In an earlier meta-analysis of 26 studies in a total of 1,058 patients with Alzheimer's disease and 932 controls, those with Alzheimer's disease had significantly higher levels of serum copper than the healthy controls [35].

Very little clinical evidence is available on the impact of copper supplementation in patients with Alzheimer's disease. One clinical trial that randomly assigned 68 patients age 50 to 80 years with mild Alzheimer's disease to supplementation with 8 mg copper daily or placebo for 12 months found no significant differences in cognition between groups [36].

Experts participating in the 2013 International Conference on Nutrition and the Brain suggested that individuals at increased risk of Alzheimer's disease using multivitamin/mineral supplements choose those that have no copper (or iron) because excessive intakes of these minerals could contribute to cognitive issues in some patients [37]. However, much more research is needed to determine whether high or low levels of serum or plasma copper are associated with Alzheimer's disease risk and whether supplements containing copper could affect Alzheimer's disease risk or symptoms.

Health Risks from Excessive Copper

Chronic exposure to high levels of copper can result in liver damage and gastrointestinal symptoms (e.g., abdominal pain, cramps, nausea, diarrhea, and vomiting) [10,38]. Copper toxicity is rare in healthy individuals who do not have a hereditary copper homeostasis defect. However, copper toxicity has been reported in people who consume water containing high levels of copper as a result of stagnant water in copper-containing pipes and fixtures as well as copper alloys in water distribution systems and household plumbing that allow copper to leach into

water [10,38]. The Environmental Protection Agency has established a recommended upper limit for copper in public water systems of 1.3 mg/L [38,39].

People with Wilson's disease, a rare, autosomal recessive disease, have a high risk of copper toxicity. Wilson's disease, which is caused by a mutation in *ATP7B*, leads to abnormally high tissue levels of copper as a result of defective copper clearance [40]. People with this disease can develop neurologic and liver damage that can result in cirrhosis [1]. Patients can also develop acute hepatitis, hemolytic crisis, and liver failure. Lifelong copper chelation therapy or high doses of zinc can prevent permanent organ damage in these patients.

The FNB has established ULs for copper from food and supplements for healthy individuals based on levels associated with liver damage [10]. The ULs do not apply to individuals who are receiving supplemental copper under medical supervision.

Table 3: Tolerable Upper Intake Levels (ULs) for Copper [10]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	None established*	None established*		
7–12 months	None established*	None established*		
1–3 years	1,000 mcg	1,000 mcg		
4–8 years	3,000 mcg	3,000 mcg		
9–13 years	5,000 mcg	5,000 mcg		
14–18 years	8,000 mcg	8,000 mcg	8,000 mcg	8,000 mcg
19+ years	10,000 mcg	10,000 mcg	10,000 mcg	10,000 mcg

* Breast milk, formula, and food should be the only sources of copper for infants.

Interactions with Copper

Copper is not known to have any clinically relevant interactions with medications.

Copper and Healthful Diets

The federal government's 2020–2025 *Dietary Guidelines for Americans* notes that "Because foods provide an array of nutrients and other components that have benefits for health, nutritional needs should be met primarily through foods. … In some cases, fortified foods and dietary supplements are useful when it is not

possible otherwise to meet needs for one or more nutrients (e.g. during specific life stages such as pregnancy)."

For more information about building a healthy dietary pattern, refer to the *Dietary Guidelines for Americans (https://www.dietaryguidelines.gov)* and the USDA's *MyPlate. (https://www.choosemyplate.gov/)*

The *Dietary Guidelines for Americans* describes a healthy dietary pattern as one that

- Includes a variety of vegetables; fruits; grains (at least half whole grains); fat-free and low-fat milk, yogurt, and cheese; and oils.

  Some vegetables, fruits, grains, and dairy products contain copper.

- Includes a variety of protein foods such as lean meats; poultry; eggs; seafood; beans, peas, and lentils; nuts and seeds; and soy products.

  Some organ meats, seafoods, and nuts and seeds are rich in copper, and other types of meats, fish, and beans contain copper.

- Limits foods and beverages higher in added sugars, saturated fat, and sodium.

- Limits alcoholic beverages.

- Stays within your daily calorie needs.

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