Cyclohexylamine

Processing

Chemical Name(s):
Cyclohexylamine

CAS Number:
108-91-8

Other Names:
CHA, Cyclohexanamine, aminocyclohexane, hexahydroxyanaline, aminohexahydrobenzene, hexahydrobenzenamine

Other Codes:
NIOSH Registry Number: GX0700000
UN/ID Number: UN2357

Summary of Advised Recommendation*

Synthetic / Non-Synthetic:	Allowed or Prohibited:	Suggested Annotation:
Synthetic	*Prohibited*	*None.*

Characterization

Composition:
$C_6H_{13}N$

Properties:
Strong fishy amine odor; colorless to yellow liquid; strong base; miscible with water and with common organic solvents: alcohol, ethers, ketones, esters, aliphatic hydrocarbons; completely miscible with aromatic hydrocarbons; soluble in chlorinated hydrocarbons, mineral oil, peanut oil, and soybean oil; molecular weight 99.17; boiling point 134.5 deg C at 760 mm Hg; melting point -17.7 deg C; specific gravity 0.8647 at 25 deg C; on distillation with water cyclohexylamine forms azeotropic mixture, boiling at 96.4 deg C at 76 mmHg; reacts with excess ammonia and zinc chloride at 350 deg C to produce alpha-picoline; reacts with organic compounds containing an active halogen atom, acid anhydrides and alkylene oxides, to replace one or both hydrogen atoms on the nitrogen atom; reacts with nitrous acid to form cyclohexanol.

How Made:
Prepared by the catalytic hydrogenation of aniline at elevated temperatures and pressures. Fractionation of the crude reaction product yields cyclohexylamine, unchanged aniline, and a high-boiling residue containing N-phenylcyclohexylamine (cyclohexylaniline) and dicyclohexylamine (Budavari, 1996). Also produced by a reaction of cyclohexanone and ammonia through reductive ammoniation. This reaction also co-produced dicyclohexylamine (Ashford, 1995).

Specific Uses:
Petititioned for use as a boiler water additive. It is also used to manufacture numerous synthetic chemicals, including insecticides, plasticizers, emulsifying agents, dyes, dry-cleaning soaps, and acid gas absorbents.

Action:
Goes into solution in boiler water and forms an azeotrope. This means that the substance cannot be separated from water by distillation or filtration and is carried over in the steam. Neutralizes carbonic acid in steam and steam condensates.

Combinations:
Used in combination with diethylaminoethanol (DEAE), morpholine, and octadecylamine (ODA) among other compounds. Often blended in proprietary mixtures that do not list solvents or carriers. It is also an inert ingredient in pesticides and has a wide range of industrial applications. Not compatible with strong oxidizers (such as chlorine, bromine, and fluorine), strong acids (such as hydrochloric, sulfuric, and nitric), acid chlorides and acid anhydrides.

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* *This Technical Advisory Panel (TAP) review is based on the information available as of the date of this review. This review addresses the requirements of the Organic Foods Production Act to the best of the investigator's ability, and has been reviewed and commented on by experts on the TAP. The substance is evaluated against the criteria found in section 2119(m) of the OFPA (7 USC 6517(m)). The information and advice presented to the NOSB is based on the technical evaluation against that criteria, and is not intended to incorporate commercial availability, socio-economic impact, or any other factor that the NOSB and the USDA may want to consider in making their decisions.*

Status

OFPA

Equipment cleaner [7 USC 6517(c)(1)(B)(i)].

Regulatory

FDA approved as a boiler water additive not to exceed 10 ppm in steam, and not approved for contact with milk and milk products [21CFR 173.310(d)].

EPA/NIEHS/Other Appropriate Sources

EPA - Cyclohexylamine (CHA) appears on the Superfund Amendments and Reauthorization Act (SARA) Title III and Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) List of Extremely Hazardous Substances (40 CFR 355 Appendix A). It is also subject to SARA reporting requirements contained in 40 CFR 311 and 40 CFR 312. Manufacturers of CHA are subject to Superfund requirements in 40 CFR 313.

The Reportable Quantity (RQ) is 10,000 lbs.
The Threshold Planning Quantity (TPQ) is 10,000 lbs.

CHA is classified as a Volatile Organic Compound (VOC) under §111 (subpart VV) of the Clean Air Act (40 CFR 60.489) and is subject to compliance with the emission standards set for VOCs.

CHA also appears on a list of priority chemicals provided by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL), established by EPA under the Federal Advisory Committee Act (FACA). This substance is one of 85 industrial chemicals and pesticides selected for development of short-term exposure levels of airborne releases that will be used by federal, state, local and private institutions when responding to emergency situations involving accidental chemical releases. Additionally, the AEGLs can be used by various organizations that are involved in chemical manufacturing, processing, storing, and transporting, or for waste remediation processes. NAC/AEGL encourages the submission of acute toxicity data or other toxicity studies on any of the substances listed (62 *Fed. Reg.* 27733).

NIEHS - National Toxicology Program database:

Acute Toxicity: (Abbreviations)

Dose	Mode	Specie	Amount	Unit
LD_{50}	ORL	RAT	710	MG/KG
LD_{50}	IPR	RAT	200	MG/KG
LD_{50}	IPR	MUS	300	MG/KG
LD_{50}	SCU	MUS	1150	MG/KG
LD_{50}	SKN	RBT	320	MG/KG
LDLO	PAR	RBT	500	MG/KG

AQTX/TLM96: 1000-100 PPM

Sax Toxicity Evaluation: Moderate via oral and inhalation routes; high via intraperitoneal routes.

Carcinogenicity: Not Available

Mutagenicity:

CYT-HMN:LEU	10 UMOL/L/5H
CYT-RAT-UNK	50 MG/KG
SPM-RAT-IPR	5 MG/KG/5D
DLT-MUS-IPR	500 MG/KG/5D-I
CYT-HAM: FBR	10 MG/L
CYT-DOM-UNK	50 MG/KG

Teratogenicity: Not Available. [See discussion below]

Standards, Regulations & Recommendations:
OSHA: Final Limit: Permissible Exposure Level (PEL) Time Weighted Average (TWA): 10 ppm [610] (Federal Register (1/19/89))

ACGIH: Threshold Limit Value (TLV) TWA 10 ppm [610]
NIOSH Criteria Document: None
NFPA Hazard Rating: Health (H): 2
Flammability (F): 3
Reactivity (R): 0
H2: Materials hazardous to health, but areas may be entered freely with full-faced mask self-contained breathing apparatus which provides eye protection (see NFPA for details).
F3: Materials which can be ignited under almost all normal temperature conditions (see NFPA for details).
R0: Materials which are normally stable even under fire exposure conditions and which are not reactive with water (see NFPA for details).

Other Toxicity Data:
Skin and Eye Irritation Data: skn-hmn 125 mg/48H SEV
Review: Toxicology Review-2

Other Data (Regulatory)
Hazard Class: 8; Subsidiary Risk: 3; Packing Group: II

Labels Required: Corrosive and Flammable liquid

Acute/Chronic Hazards:
Toxic. Causes irritation on contact. Highly toxic decomposition products. Mutagen.

Minimum Protective Clothing: If Tyvek-type disposable protective clothing is not worn during handling of this chemical, wear disposable Tyvek-type sleeves taped to your gloves.

Recommended Glove Materials: The following gloves show the best resistance based on permeation testing. It is recommended that two different glove types be used for best protection. However, if this chemical makes direct contact with your glove, or if a tear, puncture or hole develops, remove them at once. Butyl rubber (to 160 min.)

Recommended Respirator: Where the neat test chemical is weighed and diluted, wear a NIOSH-approved half face respirator equipped with a combination filter cartridge, i.e. organic vapor/acid gas/HEPA (specific for organic vapors, HCl, acid gas, SO2 and a high efficiency particulate filter).

Storage Precautions: You should store this chemical in a freezer and away from all mineral acids and bases.

Spills And Leakage: If you should spill this chemical, use absorbent paper to pick up all liquid spill material. Seal the absorbent paper, as well as any of your clothing which may be contaminated, in a vapor-tight plastic bag for eventual disposal. Wash any surfaces you may have contaminated with a strong soap and water solution. Do not reenter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned.

Disposal And Waste Treatment: You should dispose of all waste and contaminated materials associated with this chemical as specified by existing local, state and federal regulations concerning hazardous waste disposal. It is suggested that your contaminated materials should be destroyed by incineration in a special, high temperature (>2000 degrees F), chemical incinerator facility.

Emergency Procedures

Skin Contact:
IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash all affected skin areas thoroughly with soap and water.
IMMEDIATELY call a hospital or poison control center even if no symptoms (such as redness or irritation) develop.
IMMEDIATELY transport the victim to a hospital for treatment after washing the affected areas.

Inhalation:
IMMEDIATELY leave the contaminated area; take deep breaths of fresh air.
IMMEDIATELY call a physician and be prepared to transport the victim to a hospital even if no symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used; if not available, use a level of protection greater than or equal to that advised under Respirator Recommendation.

Eye Contact:
First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center.

Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. IMMEDIATELY transport the victim after flushing eyes to a hospital even if no symptoms (such as redness or irritation) develop.

Ingestion:
If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center.

Generally, the induction of vomiting is NOT recommended outside of a physician's care due to the risk of aspirating the chemical into the victim's lungs. However, if the victim is conscious and not convulsing and if medical help is not readily available, consider the risk of inducing vomiting because of the high toxicity of the chemical ingested. Ipecac syrup or salt water may be used in such an emergency. IMMEDIATELY transport the victim to a hospital. If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY transport the victim to a hospital.

Symptoms: May cause irritation on contact. Causes nausea and narcotic effects.

Firefighting:
This compound is not very flammable but any fire involving this compound may produce dangerous vapors. You should evacuate the area. All firefighters should wear full-body protective clothing and use self-contained breathing apparatuses. You should extinguish any fires involving this chemical with a dry chemical, carbon dioxide, foam, or halon extinguisher.

Other sources
US Department of Transportation - Contained on the DOT Hazardous Materials Table (59 *Fed. Reg.* 67395).

State Right-to-Know Lists: Illinois (1991), Massachussets (1994), New Jersey (1989), Pennsylvania (1989).

Status Among U.S. Certifiers
Not allowed by any U.S. Certifier. See the discussion regarding boiler water additives in the background paper Steam Generation in Organic Food Processing Systems (Steam Paper).

International
Canada – Not included in the list of permitted non-organic additives substances for organic food products (CGSB, 1999).

CODEX- Not in Annex 2, Table 4, 'Processing Aids' (FAO/WHO, 1999).

EU 2092/91 – Not in Annex VI, 'Processing Aids' (EU 2092/91).

IFOAM – Not on Appendix IV, approved processing aids and other products (IFOAM, 2000).

Japan — Not on the list of approved food additives (Woolsey, 2000).

OFPA 2119(m) Criteria

(1) *The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.*
As this is a processing material, the substance is not used in organic farming systems. Chemical interactions within a processing environment is discussed in the Steam Paper.

(2) *The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.*
See processor criteria (3) below.

(3) *The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.*
This is considered below under item (2).

(4) *The effect of the substance on human health.*
Cyclohexylamine is a severe eye, skin, and respiratory irritant, and is toxic when taken in by any route, including dermal, ingestion, inhalation, mucous membranes (IPCS, 1993). It causes second- and third-degree burns on short contact, and is very injurious to the eyes. It is strongly caustic, and inhalation can cause severe burns. Recommended protection for handling this material involves gloves, goggles, and respirators (Cheremishinoff, 1999; NTP, 2001). Some references advise wearing a self-contained breathing apparatus when handling cyclohexylamine (Toxnet, 2001). Systemic affects on humans include nausea and vomiting, anxiety, restlessness and drowsiness; spinal-type convulsions occur in rabbits (Gosselin, et.al., 1984). The LD_{50} value in rats (oral) is 156 mg/kg, and in rabbits (skin) is 277 mg/kg (Patnaik, 1992).

This is further considered in the context of the effect on nutrition (3) below as well as the consideration of GRAS and residues (5) below.

(5) *The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.*
As this is not released into the agroecosystem, there is no direct effect.

(6) *The alternatives to using the substance in terms of practices or other available materials.*
See discussion of alternatives in the Steam Paper, and the comments of the reviewers below.

(7) *Its compatibility with a system of sustainable agriculture.*
This is considered more specifically below in the context of organic handling in (6) below.

Criteria from the February 10, 1999 NOSB Meeting

A PROCESSING AID OR ADJUVANT may be used if;

1. *It cannot be produced from a natural source and has no organic ingredients as substitutes.*
CHA cannot be produced from natural sources and has no organic ingredients as substitutes. Steam can be produced from water without the addition of boiler water additives. A list of substances that are FDA approved for boiler water contact is attached. While these are not direct substitutes, these are available options. The NOSB has already recommended that several of these be listed. When considering chemical means to condition steam lines in boiler systems, the additives to the steam lines must be volatile, so that they purposely travel along with the steam. There are no known non-synthetic boiler additives that can serve this purpose. See the Steam Paper for more discussion.

2. *Its manufacture, use, and disposal do not have adverse effects on the environment and are done in a manner compatible with organic handling.*
Cyclohexylamine is made from aniline, which is a coal tar derivative (Budavari, 1996) that is regarded as highly toxic and can be absorbed into the skin in fatal amounts (Archer, 1996). The environmental impacts of coal tar production, from mining to refining, are extensive, and are beyond the scope of this review. N-phenylcyclohexylamine (cyclohexylaniline) and dicyclohexylamine are also volatile amines. Dicyclohexylamine's rat LD_{50} is 200-373 mg/kg (Greim, 1997) , which would normally be considered 'very toxic' (Gosselin, Smith, and Hodges, 1984).

   In general, volatile amines are highly reactive, and they are acknowledged to be hazardous materials to handle. Extra precautions in handling and disposal are required (Archer, 1996).

   Amines react with carbon dioxide and water to form carbamic acid (NH_2COOH). Carbamic acid is itself unstable and highly reactive in water, but readily form members of the large family of chemicals known as 'carbamates' (Streitweiser and Heathcock, 1985).

   As noted above, it is listed as an Extremely Hazardous Substance under Superfund. Disposal must be in compliance with EPA Hazardous Substance regulations.

3. *If the nutritional quality of the food is maintained and the material itself or its breakdown products do not have adverse effects on human health as defined by applicable Federal regulations.*
Cyclohexylamine (CHA) functions on steam, not on the food. It is a poison by ingestion, skin contact, and intraperitoneal routes (Lewis, 1989).

   Most of the studies on the adverse health effects of CHA are based on its properties as a metabolite of the artificial sweeteners, the cyclamates (Bopp, Sonders, and Kesterson, 1985). Sodium cyclamate directly metabolizes into CHA in all mammalian species (NRC, 1985). The rate and frequency of this conversion is a matter of scientific debate (Bopp, Sonders, and Kesterson, 1985). The FDA banned cyclamates in 1970 under the Delaney clause because it was suspected of being a carcinogen (35 *Fed. Reg.* 13644). Cyclamates in combination with saccharin and cyclohexylamine were reported to cause bladder cancer in rodents (Bryan, G.T. and E. Erturk, 1970; Price et al., 1970). Subsequent studies have failed to replicate these earlier findings (for example, Gaunt, et al., 1976; Hardy, et al., 1976). The National Research Council also concluded that there was no clear evidence that cyclamates or cyclohexylamine cause cancer (NRC, 1985).

   These studies consistently recognize and note that CHA is 20 to 50 times more toxic than cyclamates, that CHA is more biologically active than cyclamates, and that CHA consistently shows other adverse health effects not exhibited by cyclamates. A comprehensive review of the studies and a summary of the findings is contained in Bopp, Sonders, and Kesterson (1985). That review concludes that neither cyclamates nor cyclohexylamine are carcinogenic or teratogenic. More recent sources report that cyclohexylamine may be mutagenic to animal models (Patnaik, 1992) and there is evidence that it is a human mutagen (Lewis, 1989). In a number of studies, the adverse health effects of CHA were conceded, and the researchers questioned the frequency of conversion of cyclamate to CHA. In particular, studies consistently show that CHA causes testicular atrophy in test animals (Bopp, Sonders, and Kesterson, 1985; Patnaik, 1992).

4. *Its primary purpose is not as a preservative or used only to recreate/improve flavors, colors, textures, or nutritive value lost during processing except in the latter case as required by law.*
The primary use is to prevent corrosion of boiler and steam line equipment. It does not serve as a preservative, or to recreate/improve flavors, colors, textures, or nutritive value lost during processing. The use is not intended to have any technical or functional affect on the food product. The material comes into direct contact with organic foods though, which is the reason for the petition.

5. *Is Generally Recognized as Safe (GRAS) by FDA when used in accordance with Good Manufacturing Practices (GMP), and contains no residues of heavy metals or other contaminants in excess of FDA tolerances.*
The FDA does not classify cyclohexylamine as Generally Recognized as Safe (GRAS). The FDA sets a threshold for its use in steam is not to exceed 10 parts per million (ppm), and excludes use in milk and milk products (21 CFR 173.310). CHA is on the FDA Priority-Based Assessment of Food Additives (PAFA) File (CFSAN, 1998). Cyclohexylamine does not contain any heavy metals.

6. *Its use is compatible with the principles of organic handling.*
Organic standards are precautionary when evaluating synthetic substances used in food. Volatile amines in general, and cyclohexylamine in particular, do not appear to be compatible with the principles of organic handling. They are synthetic, toxic, and are not necessary to produce any food. Given the environmental impacts of the manufacturing process and the adverse health effects from exposure, they do not fit within organic principles. Food processors generated and used steam for a long time without these chemicals. Many organic food processors have already adopted viable and practical ways to address corrosion. The reviewers also comment on the availability of alternatives.

7. *There is no other way to produce a similar product without its use and it is used in the minimum quantity required to achieve the process.*
Again, culinary steam can be produced without the use of this chemical. See the Steam Paper and the reviewers' comments for further discussion.

TAP Reviewer Discussion*

Reviewer 1 [Food Science and Nutrition Professor with inspection and certification experience]
Cyclohexylamine is a neutralizing amine which acts as an azeotrope to neutralize carbonic acid produced from dissolved CO_2 in the steam which reacts with water to form the carbonic acid as the corrosive agent. It is widely used as a volatile amine type boiler additive for both its effectiveness and generally low cost . . . It has an acute oral toxicity of LD_{50} of 360 mg/kg ranking it the most toxic of cycloaliphatic amines. It is also used in the manufacture and synthesis of Siduron, a crab grass and weed control agent.

Cyclohexylamine is a major metabolite of cyclamate, a class of artificial sweeteners that was banned by the FDA. Acute LD_{50} values are 20 to 50 times lower than those of cyclamate meaning 20 to 50 times as toxic as cyclamates. The literature is replete with studies showing the toxicity of cyclohexylamine and further studies . . . have failed to confirm earlier findings. Therefore toxicity of cyclohexylamine remains controversial.

. . . *[C]*yclohexylamine is synthetic . . . manufactured from highly toxic aniline. Overall because of its potential toxicity the FDA has not approved its use as GRAS and has set a threshold for its use in steam at 10 ppm. It cannot be used in milk and dairy processing where there is direct contact with milk.

Use of cyclohexylamine on the basis of all the adverse health information provided in the scientific literature is not consistent with organic principles and practices. Its use, either by itself or with other neutralizing volatile amines, is based on its anti-corrosion properties as a boiler additive. There are many other means of reduction of steam and boiler corrosion such as boiler feed water treatments and/or installation of stainless steel steam lines. . .

Therefore on the basis of its synthetic properties, non-GRAS status, controversial worker safety and health issues I recommend that use of cyclohexylamine as a boiler additive be prohibited for all organic process operations where there is direct steam contact with food. I feel the food processing industry has a significant number of alternatives to insure steam and boiler integrity *[as well as]* energy efficiency as outlined in previous discussions.

Advised Recommendations to the NOSB
Synthetic
Prohibited
Suggested annotation: prohibited for processing operations where there is direct steam to food contact.

OMRI's information is enclosed in square brackets in italics. Where a reviewer corrected a technical point (e.g., the word should be "intravenous" rather than "subcutaneous"), these corrections were made in this document and are not listed here in the Reviewer Comments. The rest of the TAP Reviewer's comments are edited for identifying comments, redundant statements, and typographical errors. Any text removed is identified by ellipses [. . .] Statements expressed by reviewers are their own, and do not reflect the opinions of any other individual or organization.

Reviewer 2 [Consultant to organic certifiers]
Cyclohexylamine is a synthetic material. . . An equivalent substance cannot be produced from a natural source and has no substitutes that are organic ingredients. . . Cyclohexylamine is derived from aniline, which itself is highly poisonous, derived from a number of sources. Alternatively, synthesis of cyclohexylamine from cyclohexanone (see above) relies on benzene as a reaction component, and therefore also involves highly toxic materials (Budavari, 1996). . . Cyclohexylamine is heavier than air and can travel a considerable distance to a source of ignition and flash back. Its vapors form explosive mixtures with air. Vigorous reactions may occur when the amine is mixed with strong acids or oxidizers (Patnaik, 1992). . . *[C]*yclohexylamine raises significant concerns regarding its toxicological affects on humans, animals, and the environment.

The reaction of this synthetic material with organic foodstuffs may create a variety of synthetic by-products, the health implications of which are not completely known, especially over the long-term. There is no indication that addition of cyclohexylamine to the processing stream has a beneficial affect on the nutritional quality of food.

Historically, NOSB recommendations have been against the contact of any synthetic boiler additives with organic foods. All organic production and processing standards are in agreement that toxic substances should not contaminate organic foods. Organic certifiers in the United States, if they take a position at all on this issue, are consistent in repeating the prohibition recommended by the NOSB. . .

Many studies have provided assessments of the toxicity of cyclohexylamine, as a corollary to investigations made on the affect of cyclamates on mammalian species. [*The studies that show that c*]yclamates . . . could be metabolized to cyclohexylamine. Cyclohexylamine was in turn discovered to be considerably more toxic than cyclamate, the acute LD_{50} values being 20 to 50 times lower than for cyclamates, and that cyclohexylamine may be a carcinogen (Bopp, et. al, 1985). While the studies undertaken have not produced absolutely consistent results, and the carcinogenicity has not been fully reproducible, the risk involved with ingestion of cyclohexylamine (and cyclamates) remains a serious concern. . .

Live steam can be and is produced in many processing systems without the use of any boiler additives that carry over onto the food products. Boiler water can be treated in advance of use in the system by a variety of methods to soften, deionize, filter, and otherwise purify it. These steps reduce the need for addition of synthetic materials not on the National List to the boiler system. In some applications, the steam or heating system for the food may be changed to one where live steam is not the active agent, but rather heating (of food contents directly, or of steam in contact with food) is done via a heat exchange system. The wide variety and individuality of processing systems which exist is indicative of the many ways in which the full range of processed food products can be made, without the need for toxic boiler additives to be used in contact with organic foods. This reviewer does not know of any food product type that absolutely requires cyclohexylamine in steam which contacts organic food.

Justification of use of cyclohexylamine by the petitioners is based on the constraints of their particular boiler and steam systems as they currently exist, and on the financial and/or logistical challenges involved with changing those systems so as to avoid contact of the organic food by the cyclohexylamine. However, economic considerations are clearly not one of the criteria (either in OFPA or the final NOP rule) for determining the suitability of materials used in organic production systems.

History shows that quite often it has been the case that an organic operator (producer or handler) has had to make substantial changes to their system in order to be compliant with organic standards. These changes often involved redesigning of systems, practices, and techniques. In many cases, such changes resulted in the need for financial investment, as well as an investment in time. Some creativity on the part of the operator was often needed, to devise a new system. This has indeed been the case for certain processors, who made adjustments to their boiler systems or manufacturing practices in order to comply with the prohibition of contact of organic foodstuffs by synthetic boiler chemicals. The inconvenience of having to retool or readjust systems should not be the determining factor in whether or not such materials are added to the National List.

For certain processors, where organic processing events are not frequent, the boiler may be operated without the cyclohexylamine for a limited time, without significant affect on the boiler or steam line system. For these operations, no retooling may be needed; instead, a procedure can be designed whereby it is verifiable that the volatile boiler chemical has been exhausted from the system prior to handling the organic goods.

For processors who intend to process frequently enough, or for long enough run times, redesigning of the system will be necessary, in one way or another. Prohibition on the use of volatile boiler chemicals can exist without consigning processors to premature deterioration of their equipment. It is often the case in industry that the creative process involved in redesigning systems has unpredicted benefits (short- and long-term) to the operator and the environment, in terms of long-tern cost-effectiveness and sustainability; efforts in this direction should be encouraged, especially if not doing so results in a compromise of organic principles.

In fact, running boiler equipment designed for use with synthetic additives without the additives in place does lead to deterioration, and consequent lower efficiency of the system, which generally means greater energy consumption (Kohan, 1997). While greater efficiency of energy consumption seems undoubtedly to be desirable (both economically and ecologically), energy balance as a whole has not been considered as factor by the NOSB or certifiers when making determinations on the compatibility or allowability of materials or methods. To use such a factor as a criterion in the case for the volatile boiler additive is therefore inconsistent with the rest of the paradigm, and should not be a determining factor at this time.

Advised Recommendations to the NOSB
Cyclohexylamine should be deemed a synthetic, prohibited material, and not be added to the National List for any purpose.

Reviewer 3 [University staff in Food Science with inspection, consulting, and certification experience]
Cyclohexylamine (CHA) is petitioned for use as a steam additive chemical to reduce corrosion in pipes. There could be direct food contact in many processing operations when steam is used to cook or heat food, such as in a blancher, cooker, canner, or other operations. CHA has no functionality toward the food.

In the petition, page C-3 has the structure incorrect. There is no oxygen in the ring, it's a CH_2 group. . .

Response to Criteria
CHA is on the EPA List of Extremely Hazardous Substances. This would make its use of serious concern to the organic industry.

There is mixed information about this. Sodium cyclamate (from which CHA is a metabolite) was once approved as an artificial sweetener, but subsequent studies which pointed to its carcinogenesis caused it to be banned in the US. Subsequent studies seem to indicate that it isn't carcinogenic, but it has retained its banned status in the US. In spite of cyclamate's use as a sweetener, it is still categorized as an Extremely Hazardous Substance by EPA based on its irritation and fire hazards. With this mixed message, there is sufficient evidence of potential adverse effects that precautionary action does not warrant allowing its use. . .

The justification for use of CHA is no different than trying to justify the use of a synthetic herbicide like Round-Up for organic farming, just because it provides a cheaper alternative to weed control and does not leave any detectable residue. Organic handling isn't about economics or end product testing, it's the process that's critical when evaluating compatibility with organic principles. Food processors generated and used steam for a long time without these chemicals. Many organic food processors have already adopted viable and practical ways to address corrosion without the use of CHA.

There are other solutions that could be used to produce the desired result (no corrosion of piping). To summarize many of the citations reviewed, 'use of stainless steel piping completely solves the problem of corrosion.' The justification statement in the petition and the alternative control methods do not mention this as a possible solution. They do mention the costs of capital equipment and provide anecdotal evidence of the life expectancy and replacement needs should boiler water additives not be used, but provide no data to support this. There are numerous tests that can and should be performed periodically to determine the corrosion rates, (even with the use of inhibitors) to insure that equipment is being operated and maintained in a safe and efficient manner. Without confirming studies to show the differences in corrosion rates with and without the use of corrosion inhibitors, it appears that these petitioners are using anecdotal evidence to justify their continued use of cheap toxic chemicals instead of more expensive, but viable alternatives. There are several cited alternatives: stainless steel piping (suitable for all operations); discontinued use during organic processing (suitable for some operations); steam to steam heat exchanger (suitable for some operations); secondary boiler for food contact application only (suitable for all operations) that could be used. None of these are necessarily cheap, but all offer a viable alternative to the use of toxic chemicals

Advised Recommendations to the NOSB
CHA should not be approved for use as a boiler chemical for organic production.

Conclusion

The reviewers unanimously consider cyclohexylamine to be synthetic, and unanimously advise the NOSB to not add it to the National List. Use should remain prohibited in organic handling.

References

See the Steam Paper.

Cheremisinoff 1999

sical State (as normally shipped): Liquid; *Color*: Colorless; *Odor*: Sharp, hydrochloric-acid-like; pungent and irritating; **(iii) Physical and Chemical Properties** — *Physical State at 15 °C and 1 atm.*: Liquid; *Molecular Weight*: 215.6; *Boiling Point at 1 atm.*: >300, >149, >422; *Freezing Point*: (est.) <77, <25, <248; *Critical Temperature*: Not pertinent; *Critical Pressure*: Not pertinent; *Specific Gravity*: 1.23 at 20°C (liquid); *Vapor (Gas) Density*: Not pertinent; *Ratio of Specific Heats of Vapor (Gas)*: Not pertinent; *Latent Heat of Vaporization*: Not pertinent; *Heat of Combustion*: (est.) -78, -43, -1.8; *Heat of Decomposition*: Not pertinent; **(iv) Health Hazards Information** — *Recommended Personal Protective Equipment*: Acid-vapor type air respirator; rubber gloves; chemical worker goggles; other protective equipment as necessary to protect skin and eyes; *Symptoms Following Exposure*: Inhalation causes irritation of mucous membrane. Contact with eyes or skin causes severe burns. Ingestion causes severe burns of mouth and stomach; *General Treatment for Exposure*: get medical attention immediately following all exposures to this compound. INHALATION: remove from exposure; support respiration. EYES: flush with water for 15 min. SKIN: flush with water. INGESTION: give large amounts of water; *Toxicity by Inhalation (Threshold Limit Value)*: Data not available; *Short-Term Exposure Limits*: Data not available; *Toxicity by Ingestion*: Grade 2; oral LD_{50}=2,830 mg/kg (rat); *Late Toxicity*: Data not available; *Vapor (Gas) Irritant Characteristics*: Data not available; *Liquid or Solid Irritant Characteristics*: Data not available; *Odor Threshold*: Data not available.

Cyclohexylamine — (i) Chemical Designations — *Synonyms*: Amynocyclohexane; Hexahydroaniline; *Chemical Formula*: $(CH_2)_5CHNH_2$; **(ii) Observable Characteristics**— *Physical State (as normally shipped)*: Liquid; *Color*: Colorless; *Odor*: Strong fishy; **(iii) Physical and Chemical Properties** — *Physical State at 15 °C and 1 atm.*: Liquid; *Molecular Weight*: 99.18; *Boiling Point at 1 atm.*: 274.1, 134.5, 407.7; *Freezing Point*: 0.1, -17.7, 255.5; *Critical Temperature*: 648, 342, 615; *Critical Pressure*: Not pertinent; *Specific Gravity*: 0.865 at 20°C (liquid); *Vapor (Gas) Density*: Not pertinent; *Ratio of Specific Heats of Vapor (Gas)*: Not pertinent; *Latent Heat of Vaporization*: 158, 87.6, 3.67; *Heat of Combustion*: (est.) -18,000, -10,000, -420; *Heat of Decomposition*: Not pertinent; **(iv) Health Hazards Information** — *Recommended Personal Protective Equipment*: rubber gloves; chemical goggles, approved Bureau of Mines respirator for organic vapors; *Symptoms Following Exposure*: Cyclohexylamine is strongly caustic. Inhalation of vapors and contact of liquid with skin and eyes causes severe burns; *General Treatment for Exposure*: INGESTION: do NOT induce vomiting. EYES: flush with water for at least 15 min. and obtain immediate medical attention. SKIN: immediately remove contaminated clothing and flush skin with large amounts of water; *Toxicity by Inhalation (Threshold Limit Value)*: 300 mg/m^3; *Short-Term Exposure Limits*: Data not available; *Toxicity by Ingestion*: Grade 3; LD_{50}50 to 500 mg/kg; *Late Toxicity*: Produced cancer of the bladder in the rat; *Vapor (Gas) Irritant Characteristics*: Vapor is moderately irritating such that personnel will not usually tolerate moderate or high vapor concentrations; *Liquid or Solid Irritant Characteristics*: Severe skin irritant. Causes second- and third-degree burns on short contact; very injurious to the eyes; *Odor Threshold*: Data not available.

Cyclopentane — (i) Chemical Designations — *Synonyms*: Pentamethylene; *Chemical Formula*: C_5H_{10}; **(ii) Observable Characteristics** — *Physical State (as normally shipped)*: Liquid; *Color*: Colorless; *Odor*: Like gasoline; mild, sweet; **(iii) Physical and Chemical Properties** — *Physical State at 15 °C and 1 atm.*: Liquid; *Molecular Weight*: 70.1; *Boiling Point at 1 atm.*: 120.7, 49.3, 322.5; *Freezing Point*: -137.0, -93.9, -179.3; *Critical Temperature*: 461.5, 238.6, 511.8; *Critical Pressure*: 654, 44.4, 4.51; *Specific Gravity*: 0.74 at 20°C (liquid); *Vapor (Gas) Density*: 2.4; *Ratio of Specific Heats of Vapor (Gas)*: 1.1217; *Latent Heat of Vaporization*: 179, 94, 3.9; *Heat of Combustion*: -19,990, -11,110, -465; *Heat of Decomposition*: Not pertinent; **(iv) Health Hazards Information** — *Recommended Personal Protective Equipment*: Hydrobarbon canister, supplied air, or hose mask; rubber or plastic gloves; chemical goggles or face shield; *Symptoms Following Exposure*: Inhalation causes dizziness, nausea, and vomiting; concentrated vapor may cause unconsciousness and collapse. Vapor causes slight smarting of eyes. Contact with liquid causes irritation of eyes and may irritate skin if allowed to remain. Ingestion causes irritation of stomach. Aspiration produces severe lung irritation and rapidly developing pulmonary edema; central nervous excitement followed by depression; *General Treatment for Exposure*: INHALATION: remove to fresh air; if breathing stops, apply artificial respiration and administer oxygen. EYES: flush with water for at least 15 min.; call a physician. SKIN: flush well with water, then wash with soap and water. INGESTION: do NOT induce vomiting; guard against aspiration into lungs. ASPIRATION: enforce bed rest; give oxygen; get medical attention; *Toxicity by Inhalation (Threshold Limit Value)*: Data not available; *Short-Term Exposure Limits*: 300 ppm

References and Notes

1. T. K. Kelly, W. F. Lindqvist, M. D. Muir, *Science* 165, 283, and cover picture (1969).
2. D. G. Coates, *Proceedings of the Second Annual Scanning Electron Microscopy Symposium* (IIT Research Institute, Chicago, 1969), p. 29; A. M. B. Shaw, G. R. Booker, D. G. Coates, *J. Sci. Instr.* Ser. 2, 2, 243 (1969).
3. K. F. J. Heinrich, "Scanning Electron Probe Microanalysis," *Nat. Bur. Std. (U.S.) Tech. Note 278* (Feb. 1967), p. 6.

4. We gratefully acknowledge the expertise and efforts of L. Marzetta of the Measurement Engineering Division, NBS, who has been of great help in constructing the necessary electronic devices.

28 October 1969; revised 10 December 1969 ■

Bladder Tumors in Rats Fed Cyclohexylamine or High Doses of a Mixture of Cyclamate and Saccharin

Abstract. *Papillary transitional cell tumors were found in the urinary bladders in 8 rats out of 80 that received 2600 milligrams per kilogram of body weight per day of a mixture of sodium cyclamate and sodium saccharin (10 : 1) for up to 105 weeks. From week 79 on, several of these rats received cyclohexylamine hydrochloride (125 milligrams per kilogram per day, the molecular equivalent of the conversion of about 10 percent of the cyclamate dosage to cyclohexylamine) in addition to the sodium cyclamate and sodium saccharin. In another study in which 50 rats were fed daily 15 milligrams of cyclohexylamine sulfate per kilogram of body weight for 2 years, eight males and nine females survived. One of the eight males had a tumor of the urinary bladder: In neither study were bladder tumors found in the control rats or in rats treated with lower doses of the compounds.*

Numerous requests have been made for the information which was presented to the National Academy of Sciences–National Research Council (NAS–NRC) ad hoc Committee on Nonnutritive Sweeteners on 17 October 1969, and which led to the order by the Secretary of Health, Education and Welfare that cyclamates be removed from the list of substances generally recognized as safe (GRAS). In this preliminary report we present the pertinent experimental findings in the context of some relevant historical information.

The enactment of the Food Additives Amendment of 1958 made it necessary to establish at least a partial list of substances generally recognized as safe since such substances generally were exempted from the application of this statute. Food and Drug Administration (FDA) scientists prepared such a list, which included cyclamates, and this was sent to over 900 qualified scientists for comment. Of the 355 scientists who responded, only one commented on cyclamates stating that he was unfamiliar with the data on these sweeteners. Thus, cyclamates were included in the published list, as set forth in the Code of Federal Regulations (Section 121.101).

In 1962, the Food and Nutrition Board of the NAS–NRC issued a revised policy statement which said that artificial sweeteners could be safely used in limited amounts as a nonnutritive substitute for sugar in special purpose foods.

In 1965 and again in September 1967, scientists of the FDA reexamined all available information about cyclamates and concluded that there was no evidence that the amounts of cyclamates then being used presented a hazard to health. In 1967, the joint FAO/WHO Expert Committee on Food Additives established an acceptable daily intake of 50 mg of cyclamate per kilogram of body weight. In 1968, the NAS–NRC recommended the limitation of daily intake to be 70 mg per kilogram of body weight. On the basis of these two reviews, in April 1969, the FDA proposed steps to achieve revised product labeling that would limit the daily intake to the level recommended by WHO.

The above reviews included an examination of studies in which rats were fed diets containing 1 and 5 percent saccharin or sodium cyclamate for 2 years. These compounds produced no effects at the lower dose and no distinct toxic effects at the high dose (*1*). Toxicological studies in rats fed diets containing 1 and 2 percent sodium cyclamate for periods up to 11 months indicated no significant adverse effects of this compound (*2*).

Allen *et al.* (*3*) reported in 1957 that surgical implantation of pellets containing 4 parts of cholesterol and 1 part of saccharin into the urinary bladder of mice induced one papilloma and three carcinomas of the bladder among 13 animals that survived 40 to 52 weeks. In 1966, a similar study with sodium cyclamate was initiated by one of us (J.M.P.) at the University of Wisconsin. On 5 June 1969, a preliminary verbal report (*4*) of this study was given to Abbott Laboratories, stating that a significant incidence of bladder tumors had been found in white Swiss mice in two separate experiments with the pellet implantation technique. Representatives of Abbott Laboratories had several discussions about these findings with representatives of the National Cancer Institute and the Food and Drug Administration during June and July. It was the judgment of all concerned that tests for carcinogenicity by the pellet implantation technique (*3*) were not suitable for evaluating the hazard of orally ingested compounds. A similar position regarding data obtained by this technique had been taken by the NAS–NRC ad hoc Committee on Nonnutritive Sweeteners in 1968. Plans for additional toxicity studies of cyclamates, cyclohexylamine (CHA), and saccharin were then agreed upon. It was also decided to pay special attention to the urinary bladders of rats in two toxicity studies sponsored by Abbott Laboratories which had been initiated in 1967 and were nearing completion.

One of the last-mentioned experiments, conducted at Industrial Bio-Test Laboratories, Northbrook, Illinois, was a 2-year toxicity study of cyclohexylamine in rats which was designed to ascertain whether or not the CHA which could be present in minute amounts in commercial cyclamates might be toxic. Charles River strain albino rats in groups of 25 males (125 g) and 25 females (123 g) were given daily doses of either 0, 0.15, 1.5 or 15.0 mg of cyclohexylamine sulfate per kilogram of body weight. During the first year of the study, there was only a slight depression in the weight gain curves observed in male animals fed the highest dose (*5*). There were no significant differences between test and control animals as to food consumption, mortality, blood chemistry, or hematologic parameters. At the end of 2 years, eight males and nine females were alive in the high dose group. There were 13 to 16 survivors in each of the other three groups at the end of the study. No drug-related changes were found in any of the organs examined except in the urinary bladder. A bladder tumor was found in one of the eight male survivors in the high dose group which was diagnosed as invasive transitional cell carcinoma, grade 2. The tumor did not invade the muscular wall of the bladder, and no metastatic lesions

Table 1. Summary of the preliminary data obtained in the long-term feeding study of sodium cyclamate and sodium saccharin (C/S). At the 79th week groups B, C, and D were each divided into two subgroups each containing approximately half the surviving number of converters and nonconverters. Subgroups 1 and 2 continued to receive C/S at the stated dose and subgroup 2 received in addition the indicated dose of CHA (the molecular equivalent of the conversion of about 10 percent of the cyclamate to CHA).

Group	Daily dose (mg/kg day)		No. of animals alive at week								No. converters†/ No. tested		No. tumors‡	
			0		56*		78		104					
	C/S	CHA	M	F	M	F	M	F	M	F	M	F	M	F
A	0	0	35	45	25	35	20	35	13	26			0	0
B	500	25	35	45	25	35	20	30	10	19	11/23	5/33	0	0
C	1120	56	35	45	25	35	20	31	8	23	9/24	9/32	0	0
D	2500	125	35	45	25	35	20	30	12	22	23/25	32/35	7	1

* Ten males (M) and ten females (F) died or were killed for interim study by the 56th week. There was one death in each group except for group B females (none) and group D males (two). † Rats excreting CHA in the urine in amounts equivalent to more than 0.1 percent of the cyclamate fed (see text). ‡ Urinary bladder tumors agreed upon by all of the pathologists on the basis of the slides available to date. Four to eight of these tumors were diagnosed as carcinomas by different pathologists.

were present. Spontaneous bladder tumors have never been recorded in control rats at Industrial Bio-Test Laboratories (*5*) or at Abbott Laboratories and are reported to be very rare (*6*).

The second experiment, conducted at Food and Drug Research Laboratories, Maspeth, N.Y., was a 2-year toxicity study of a 10 : 1 mixture of sodium cyclamate and sodium saccharin (C/S) which was added to the diet of Wistar strain rats in concentrations providing a daily intake of 0, 500, 1120, or 2500 mg per kilogram of body weight (Table 1). The concentrations required to provide the stated daily doses of the mixture were determined from data obtained by biweekly weighing of the animals and biweekly measurements of their food intake. The rats were maintained throughout the 2-year period in individual cages in air-conditioned and humidity-controlled quarters, with water and food freely available.

During this study many of the rats were found to convert cyclamate to cyclohexylamine (*7*). The rats were considered to convert cyclamate to cyclohexylamine if more than 0.1 percent of the cyclamate was accounted for as urinary CHA. The extent to which individual rats converted (or whether they converted) was variable. The maximum conversion rate was 12.6 percent (*7*).

In the 79th week, one-half of the animals in each of the treated groups were given supplemental amounts of cyclohexylamine hydrochloride mixed in the diet and calculated (as the base) to provide daily intakes of 25, 56, or 125 mg per kilogram of body weight. All major organs and tissues, including the urinary bladder, were examined histologically in the surviving animals as well as in those animals that died or were killed in the course of the study. Among the 240 rats receiving C/S, seven males and one female of the group fed 2500 mg per kilogram per day showed papillary tumors of the urinary bladder (Table 1) which were diagnosed by seven pathologists (*8*). In all but one instance, the tumors developed in rats that had been found to convert cyclamate to CHA. There were three bladder tumors in animals that received supplemental CHA and five in those that did not. Macroscopically, tumors were seen in only two animals. Of the eight tumors, four to eight were diagnosed as carcinomas by the different pathologists. No gross bladder calculi were found in the eight rats with tumors. Three of the tumors were found between weeks 78 and 83, and the remaining tumors were found in animals which were killed between 100 and 105 weeks of the study.

On 8 October 1969, Abbott Laboratories was first notified by telephone of the presence of bladder lesions in rats fed the C/S mixture. On 9 October Abbott pathologists observed the presence of bladder tumor in one of the rats fed CHA. On 13 October Abbott representatives reviewed the microscopic slides and other data from the study of the C/S mixture at Food and Drug Research Laboratories and on the same day reported the findings to scientists of the National Cancer Institute. On 14 October these findings were discussed in a joint meeting of representatives of Abbott Laboratories, the National Cancer Institute, FDA, and the Department of Health, Education and Welfare, and it was decided to report the findings to the NAS–NRC ad hoc Committee on Nonnutritive Sweeteners. The slides of the urinary bladders of the rats from the two studies were reviewed on 15 and 16 October by additional staff and consultant pathologists of the National Cancer Institute. All the available data from these experiments were presented on 17 October to the NAS–NRC Committee which recommended the removal of cyclamates from the GRAS list.

The development of bladder neoplasms had not been reported in other species or in other strains of rats fed cyclamate or saccharin. There is no evidence that the use of cyclamate or saccharin has caused cancer in man, malformations in children, or any other abnormality in humans other than a rare skin hypersensitivity. However, in view of the requirements of the Delaney clause of the Food Additives Amendment, the removal of cyclamates from the classification of substances generally recognized as safe resulted in the prohibition of their use in general purpose food products.

J. M. PRICE, C. G. BIAVA
Abbott Laboratories, North Chicago, Illinois
B. L. OSER, E. E. VOGIN
Food and Drug Research Laboratories, Maspeth, New York
J. STEINFELD
Department of Health, Education, and Welfare, Washington, D.C.
H. L. LEY*
Food and Drug Administration, Washington, D.C.

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1. O. G. Fitzhugh, A. A. Nelson, J. P. Frawley, *J. Amer. Pharm. Ass.* **40**, 583 (1951).
2. J. D. Taylor, R. K. Richards, R. G. Wiegand, M. S. Weinberg, *Food Cosmet. Toxicol.* **6**, 313 (1968).
3. M. J. Allen, E. Boyland, C. E. Dukes, E. S. Horning, J. G. Watson, *Brit. J. Cancer* **11**, 212 (1957).
4. G. T. Bryan, personal communication.
5. J. C. Calandra, personal communication.
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8. C. G. Biava and U. Saffiotti; G. E. Cox and S. S. Sternberg, Food and Drug Research Laboratories; R. W. O'Gara and K. C. Snell, Laboratory of Pathology of the National Cancer Institute; G. H. Friedell, Department of Pathology, Boston University, a consultant on bladder tumors for the National Cancer Institute.

* Former commissioner, Food and Drug Administration, Washington.

24 November 1969

604 Triethylenetetramine 604

N,N'-Bis(2-aminoethyl)ethylenediamine, 3,6-Diazaoctane-1,8-diamine

Toxicity Rating: 3. Triethylenetetramine is a slightly viscous liquid polyamine similar to but less volatile than diethylenetriamine (see above). A strong organic base and chelating agent used in the synthesis of detergents, softeners and dyestuffs, the manufacture of pharmaceuticals, and the vulcanization of rubber. It is moderately toxic by ingestion, percutaneous absorption and vapor inhalation with an oral LD_{50} of 4.34 gm./kg. in rats and a dermal LD_{50} of 0.82 gm./kg. in rabbits. Respiratory irritation and erythema, edema, and itching of the face have occurred in workers exposed to hot vapors. It is a primary skin irritant, slightly less active as a sensitizer and eye irritant than the lower polyamine homologues.

Ref.: Beard and Noe, 1981.

605 Tetraethylenepentamine 605

N-(2-Aminoethyl)-*N'*-(2-((2-aminoethyl)amino)ethyl-1,2-ethanediamine, 1,4,7,10,13-Pentaazatridecane

Toxicity Rating: 3. An aliphatic polyamine with industrial applications. Toxicity to animals is similar to that of triethylenetetramine (see above). Rat oral LD_{50} is 3.99 gm./kg. Single dose rabbit dermal LD_{50} is 0.66 gm./kg. Produces intense skin irritation and moderate eye injury in rabbits but not so severe as lower homologues (see above).

606 Ethylenimine 606

Azacyclopropane, Aziridine, Dimethylenimine

Toxicity Rating: 5. Used as an intermediate in organic syntheses, ethylenimine (and presumably *N*-ethylethylenimine) is a highly reactive, strongly alkaline compound with an appreciable vapor pressure. Skin contact produces painless but severely necrotizing burns. Human exposure to vapor concentrations above 100 ppm causes respiratory tract irritation and inflammation, but symptoms may be delayed several hours. Presumably severe exposures might result in an overwhelming pulmonary edema. It is a potent lacrimator and emetic. Signs and symptoms include tearing and burning of the eyes, sore throat, vomiting, coughing (which may persist for weeks or months), and a slowly healing dermatitis. Hematologic effects include transient polycythemia, leukocytosis and eosinophilia. Hemorrhagic congestion of all internal organs occurs in high level exposures of experimental animals. In man and especially in animals renal damage has been observed, including albuminuria and hematuria. For the management of chemical injuries to the lungs, see Nitrogen Oxides in Section III.

Ref.: Walpole et al., 1954; Weightman and Hoyle, 1964.

607 Octadecylamine 607

Toxicity Rating: 3. Eighteen-carbon straight-chain amine sometimes used as an anticorrosive agent in live steam lines. Toxicity rating based on studies in mice and rats. Rats have tolerated dietary levels of 500 ppm for 2 years without signs of toxicity or pathologic changes. At levels of 3000 ppm for from 89 to 209 days, anorexia, weight loss and some histologic changes in mesenteric lymph nodes, gastrointestinal mucosa and liver. Said to be a primary skin sensitizer.

Ref.: Deichmann et al., 1958; MacDonald et al., 1962.

608 Dicyclohexylamine 608

Dodecahydrodiphenylamine

Toxicity Rating: 4. In animals somewhat more toxic than cyclohexylamine, and unlike the latter it can be absorbed in dangerous amounts through skin. Rabbits which ingest it die in convulsions. The acetate is said to produce a pressor response in dogs. See Cyclohexylamine below.

Ref.: McOmie and Anderson, 1949.

609 Cyclohexylamine 609

Hexahydroaniline, Aminocyclohexane

Toxicity Rating: 4. Numerous industrial uses include the manufacture of rubber, insecticides, corrosion inhibitors, plasticizers, etc. A minor metabolite of cyclamates in man and other species. The free amine has a caustic action on skin and mucous membranes. Systemic effects in man include nausea and vomiting, anxiety, restlessness and drowsiness. Spinal-type convulsions occur in rabbits. In mice cyclohexylamine has typical sympathomimetic effects like amphetamine with hyperpnea, hyperthermia, increased metabolic rate and a degree of lethality that is dependent on ambient temperature and crowding. Chlorpromazine, reserpine and phenoxybenzamine protect mice against death.

See also: Amphetamine, *Reference Congener in Section III.*

Ref.: Carswell and Morrill, 1937; Lee and Dixon, 1972; Watrous and Schulz, 1950.

CAS: 103-95-7

EHYDE

w: 190.31

quid; strong, floral odor. D: ndex: 1.503-1.508. Sol in propylene glycol, glycerin.

◇ CYCLAMAL ◇ FEMA No. 2743
HYLHYDROCINNAMIC ALDEHYDE
HYLPHENYLPROPYL ALDEHYDE
PYLHYDROCINNAMALDEHYDE
ROPYLPHENYL)PROPION-

g agent.

ious.

AS when used at a level not amount reasonably required intended effect.

E: Moderately toxic by inges- skin irritant. When heated to emits acrid smoke and irritating

A and CODEN

48H MLD FCTXAV 12,385,74
10 mg/kg FCTXAV 2,327,64

CAS: 110-82-7

NE

mw: 84.18

ss, mobile liquid; pungent odor. 80.7°, fp: 4.6°, flash: p: 1.4°F, el: 1.3%, uel: 8.4%, d: 0.7791 ign temp: 473°F, vap press: 100 vap d: 2.90.

SANO (ITALIAN) ◇ CYCLOHEXAAN
OHEXAN (GERMAN) ◇ CYKLOHEKSAN
AHYDROBENZENE ◇ HEXAMETHYLENE
ENE ◇ RCRA WASTE NUMBER U056

D:

or diluent.

Various.

FDA - 21CFR 73.

Right-To-Know List.

TWA 300 ppm ACGIH TL
om DOT Classification: Flamm
Label: Flammable Liquid

Lewis (1989) Food Additives Handbook

SAFETY PROFILE: Poison by intravenous route. Moderately toxic by ingestion. A systemic irritant by inhalation and ingestion. A skin irritant. Mutagenic data. Flammable liquid. Dangerous fire hazard when exposed to heat or flame; can react with oxidizing materials. Moderate explosion hazard in the form of vapor when exposed to flame. When mixed hot with liquid dinitrogen tetraoxide an explosion resulted. To fight fire, use foam, CO_2, dry chemical, spray, fog. When heated to decomposition it emits acrid smoke and fumes.

TOXICITY DATA and CODEN

skn-rbt 1548 mg/2D-I JIHTAB 25,199,43
dnd-esc 10 μmol/L MUREAV 89,95,81
orl-rat LD50:29820 mg/kg JIHTAB 25,415,43
ivn-rbt LDLo:77 mg/kg JPMRAB 3,1,28

CPF000 CAS: 622-45-7

CYCLOHEXYL ACETATE

DOT: 2243

mf: $C_8H_{14}O_2$ mw: 142.22

PROP: Pale yellow liquid; fruity odor. Bp: 177°, d: 0.996, vap d: 4.9, flash p: 136°F, autoign temp: 633°F.

SYNS: CYCLOHEXANOL ACETATE ◇ CYCLOHEXANOLAZETAT (GERMAN) ◇ CYCLOHEXANYL ACETATE

USE IN FOOD:

Purpose: Flavoring agent.

Where Used: Baked goods, beverages, candy, ice cream.

Regulations: FDA - 21CFR 172.515. Use at a level not in excess of the amount reasonably required to accomplish the intended effect.

DOT Classification: Flammable or Combustible Liquid; Label: Flammable Liquid

SAFETY PROFILE: Moderately toxic by subcutaneous route. Mildly toxic by ingestion and skin contact. Human systemic effects by inhalation: conjunctiva irritation and unspecified respiratory system changes. A systemic irritant to humans. Flammable when exposed to heat or flame. When heated to decomposition it emits acrid smoke and irritating fumes.

TOXICITY DATA and CODEN

skn-rbt 500 mg/24H MOD FCTXAV 17,695,79
[illegible]-hmn TCLo:3000 mg/m³/45M:IRR
AIHAAP 39,260,13
orl-rat LD50:6730 mg/kg TXAPA9 28,313,74
skn-rbt LD50:10 g/kg TXAPA9 28,313,74

CPF500 CAS: 108-91-8

CYCLOHEXYLAMINE

DOT: 2357

mf: $C_6H_{13}N$ mw: 99.20

PROP: Liquid; strong, fishy odor. Mp: −17.7°, bp: 134.5°, flash p: 69.8°F, d: 0.865 @ 25°/25°, autoign temp: 560°F, vap d. 3.42.

SYNS: AMINOCYCLOHEXANE ◇ AMINOHEXAHYDROBENZENE ◇ CHA ◇ CYCLOHEXANAMINE ◇ HEXAHYDROANILINE ◇ HEXAHYDROBENZENAMINE

USE IN FOOD:

Purpose: Boiler water additive.

Where Used: Various.

Regulations: FDA - 21CFR 173.310. Limitation of 10 ppm in steam and excluding use of such steam in contact with milk and milk products.

IARC Cancer Review: Animal No Evidence IMEMDT 22,55,80. EPA Extremely Hazardous Substances List. EPA Genetic Toxicology Program.

ACGIH TLV: TWA 10 ppm (skin) DFG MAK: 10 ppm (40 mg/m³) DOT Classification: Flammable Liquid; Label: Flammable Liquid, Corrosive; Flammable or Combustible Liquid; Label: Flammable, Corrosive

SAFETY PROFILE: A poison by ingestion, skin contact, and intraperitoneal routes. Moderately toxic by subcutaneous and parenteral routes. An experimental teratogen. Other experimental reproductive effects. Severe human skin irritant. Can cause dermatitis; convulsions. Human mutagenic data. Flammable or combustible liquid. Dangerous fire hazard when exposed to heat, flame, or oxidizers. To fight fire, use alcohol foam, CO_2, dry chemical. When heated to decomposition it emits toxic fumes of NO_x.

TOXICITY DATA and CODEN

skn-hmn 125 mg/48H SEV AMIHBC 5,311,52
cyt-hmn:leu 10 μmol/L/5H MUREAV 39,1,76
hma-mus/leu 450 mg/kg/3D MUREAV 31,5,75
orl-rat TDLo:5600 mg/kg (4W male):REP FCTXAV 19,291,81
orl-mus TDLo:600 mg/kg (6-11D preg):TER SEIJBO 11,51,71

over

orl-mus TDLo:120 mg/kg (6-11D preg):TER SEIJBO 11,51,71
orl-rat LD50:156 mg/kg SKEZAP 14,542,73
skn-rbt LD50:277 mg/kg AIHAAP 30,470,69

CPQ625 CAS: 100-88-9
N-CYCLOHEXYLSULPHAMIC ACID

mf: $C_6H_{13}NO_3S$ mw: 179.26

PROP: Crystals; sweet-sour taste. Mp: 169-170°. Fairly strong acid. Very sparingly soluble in water. Slowly hydrolyzed by hot water.

SYNS: CYCLAMATE ◇ CYCLAMIC ACID ◇ CYCLOHEXANESULPHAMIC ACID ◇ CYCLOHEXYLAMIDOSULPHURIC ACID ◇ CYCLOHEXYLAMINESULPHONIC ACID ◇ CYCLOHEXYLSULFAMIC ACID (9CI) ◇ CYCLOHEXYLSULPHAMIC ACID ◇ HEXAMIC ACID ◇ SUCARYL ◇ SUCARYL ACID

USE IN FOOD:

Purpose: Nonnutritive sweetener.

Where Used: Prohibited from foods.

Regulations: FDA - 21CFR 189.135. Prohibited from direct addition or use in human food.

SAFETY PROFILE: Poison by intravenous route. Mildly toxic by ingestion. A human carcinogen by ingestion (bladder tumors and hematuria). When heated to decomposition it emits toxic fumes of SO_x and NO_x.

TOXICITY DATA and CODEN

orl-man TDLo:22 g/kg/77W-C:CAR,KID JOURAA 118,258,77
orl-man TD :131 g/kg/5Y-C:CAR,KID JOURAA 118,258,77
orl-man TD :164 g/kg/6Y-C:CAR,KID JOURAA 118,258,77
orl-rat LD50:12 g/kg AJMSA9 225,551,53

CPS000 CAS: 115-25-3
CYCLOOCTAFLUOROBUTANE

DOT: 1976
mf: C_4F_8 mw: 200.03

PROP: Colorless, odorless gas. Bp: −6.04°, mp: −41.4°, d (liquid): 1.513 @ −70°F.

SYNS: FC-C 318 ◇ FREON C-318 ◇ HALOCARBON C-138 ◇ OCTAFLUOROCYCLOBUTANE (DOT) ◇ PERFLUOROCYCLOBUTANE ◇ PROPELLANT C318 ◇ R-C 318

USE IN FOOD:

Purpose: Aerating agent, propellant.

Where Used: Foamed food products, sprayed food products.

Regulations: FDA - 21CFR 173.360.

EPA Genetic Toxicology Program.

DOT Classification: Nonflammable Gas; Label: Nonflammable Gas

SAFETY PROFILE: Mildly toxic by ingestion and inhalation. Can cause slight transient effects at high concentrations. No anesthesia or central nervous system effects. Nonflammable Gas. Mutagenic data. When heated to decomposition it emits highly toxic fumes of F^-.

TOXICITY DATA and CODEN

sln-dmg-ihl 99 pph/10M ENVRAL 7,275,74

CQI000 CAS: 99-87-6
p-CYMENE

DOT: 2046
mf: $C_{10}H_{14}$ mw: 134.24

PROP: Colorless to pale yellow liquid; odorless. Mp: −68.2°, bp: 176°, lel: 0.7%, @ 100°, ULC: 30-35, flash p: 117°F (CC), d: 0.853, refr index: 1.489, autoign temp: 817°F, vap d: 4.62, vap press: 1 mm @ 17.3°, flash p: (technical) 127°F, uel (technical): 5.6%. Found in nearly 100 volatile oils including lemongrass, sage, thyme, coriander, star anise, and cinnamon (FCTXAV 12,385,74). Sol in alc, ether, acetone, benzene.

SYNS: CAMPHOGEN ◇ CYMENE ◇ CYMOL ◇ DOLCYMENE ◇ FEMA No. 2356 ◇ 4-ISOPROPYL-1-METHYLBENZENE ◇ p-ISOPROPYLTOLUENE ◇ p-METHYL-CUMENE ◇ p-METHYLISOPROPYL BENZENE ◇ 1-METHYL-4-ISOPROPYLBENZENE ◇ PARACYMENE ◇ PARACYMOL

USE IN FOOD:

Purpose: Flavoring agent.

Where Used: Various.

Regulations: FDA - 21CFR 172.515. Use at a level not in excess of the amount reasonably required to accomplish the intended effect.

DOT Classification: Flammable or Combustible Liquid; Label: Flammable Liquid

SAFETY PROFILE: Mildly toxic by ingestion. Humans sustain central nervous system effects at low doses. Mutagenic data. A skin irritant. Flammable or combustible liquid. Explosion Hazard: Slight in the form of vapor. To fight fire, use foam, CO_2, dry chemical. When heated to decomposition it emits acrid smoke and fumes.

(NFPA 1986; NIOSH 1984, Suppl. 1985): (open cup) −1°C (30°F) (Merck 1989), 7°C (45°F) (Scherberger et al. 1960); vapor pressure 82 torr at 20°C; vapor density 3.0 (air = 1); the vapor is heavier than air and can travel some distance to a source of ignition and flash back; autoignition temperature 312°C (594°F); fire-extinguishing agent: dry chemical, CO_2, or "alcohol" foam; use water to keep fire-exposed containers cool and to flush and dilute any spill.

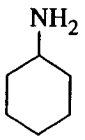
n-Butylamine forms explosive mixtures with air in the range 1.7–9.8% by volume in air. Its reactions with strong acids or oxidizers can be vigorous. Contact with acrolein may cause base-catalyzed polymerization of the latter, which is highly exothermic. *n*-Butylamine may exhibit violent reactions, characteristic of lower aliphatic primary amines (see Section 8.3).

8.7 CYCLOHEXYLAMINE

DOT Label: Flammable Liquid, Corrosive, UN 2357

Formula $C_6H_{11}NH_2$; MW 99.20; CAS [108-91-8]

Structure:



an alicyclic amine

Synonyms: cyclohexanamine; hexahydrobenzenamine; aminocyclohexane; hexahydroaniline

Uses and Exposure Risk

Cyclohexylamine is used in the manufacture of a number of products, including plasticizers, drycleaning soaps, insecticides, and emulsifying agents. It is also used as a corrosion inhibitor and in organic synthesis.

Physical Properties

Colorless or yellowish liquid with a strong fishy, amine odor; density 0.8645 at 25°C; boils at 134.5°C; solidifies at −17.7°C; miscible with water and most organic solvents; forms an azeotropic mixture with water containing 44% cyclohexylamine, which boils at 96.5°C; strongly basic.

Health Hazard

Cyclohexylamine is a severe irritant to the eyes, skin, and respiratory passage. Skin contact can produce burns and sensitization; contact of the pure liquid or its concentrated solutions with the eyes may cause loss of vision.

The acute oral and dermal toxicity of cyclohexylamine was moderate in test subjects. The toxic effects include nausea, vomiting, and degenerative changes in the brain, liver, and kidney. Inhalation of its vapors at high concentrations may cause a narcotic effect.

LD_{50} value, oral (rats): 156 mg/kg
LD_{50} value, skin (rabbits): 277 mg/klg

Cyclohexylamine may be mutagenic, the test for which has so far given inconclusive results. Administration of this compound in animals produced a reproductive effect, including embryotoxicity and a reduction in male fertility. Intraperitoneal injection of the amine in rats caused a dose-dependent increase in chromosomal breaks. Roberts and co-workers (1989) studied the metabolism and testicular toxicity of cyclohexylamine (a metabolite of cyclamate) in rats and mice. Chronic dietary administration of 400 mg/kg/day for 13 weeks showed decrease in organ weights, histological changes, and testicular atrophy in both the Wistar and DA rats, but to a widely varying extent, while mice showed no evidence of testicular damage.

There is no evidence of carcinogenicity in animals or humans caused by cyclohexylamine.

Exposure Limit

TLV-TWA 10 ppm (~40 mg/m³) (ACGIH).

Fire and Explosion Hazard
Flammable liquid; flash point (open cup) 32°C (90°F); autoignition temperature 293°C (560°F); vapor density 2.4 (air = 1); the vapor is heavier than air and can travel a considerable distance to a source of ignition and flash back. Fire-extinguishing agent: dry chemical, CO_2, or "alcohol" foam; water may be used to flush and dilute any spill and to keep fire-exposed containers cool.

Cyclohexylamine vapors form explosive mixtures with air; explosive limits data are not available. Vigorous reactions may occur when the amine is mixed with strong acids or oxidizers.

8.8 DIMETHYLAMINE

EPA Classified Toxic Waste, RCRA Waste Number U092; DOT Label: Flammable Gas/Flammable Liquid (Aqueous), UN 1032, UN 1160

Formula C_2H_6NH; MW 45.10; CAS [124-40-3]

Structure: $CH_3—NH—CH_3$, a primary amine

Synonym: *N*-methylmethanamine

Uses and Exposure Risk
Dimethylamine is used in the manufacture of *N*-methylformamide, *N*-methylacetamide, and detergent soaps; in tanning; and as an accelerator in vulcanizing rubber. It is commercially sold as a compressed liquid in tubes or as a 33% aqueous solution.

Physical Properties
Colorless gas with a pungent fishy ammoniacal odor; liquefies at 7°C; freezes at −96°C; density of liquid 0.680 at 0°C; highly soluble in water, soluble in alcohol and ether; aqueous solution strongly alkaline.

Health Hazard
Dimethylamine is a strong irritant to the eyes, skin, and mucous membranes. Spill of liquid into the eyes can cause corneal damage and loss of vision. Skin contact with the liquid can produce necrosis. At sublethal concentrations inhalation of dimethylamine produced respiratory distress, bronchitis, pneumonitis, and pulmonary edema in test animals. The acute oral toxicity was moderate, greater than for monomethylamine.

LC_{50} value, inhalation (rats): 4540 ppm/6 hr
LD_{50} value, oral (mice): 316 mg/kg

Buckley and co-workers (1985) have investigated the inhalation toxicity of dimethylamine in F-344 rats and B6C3F1 mice. Animals exposed to 175 ppm for 6 hours/day, 5 days/week for 12 months showed significant lesions in the nasal passages. Rats developed more extensive olfactory lesions than did mice. The study indicated that olfactory sensory cells were highly sensitive to dimethylamine. Even at a concentration of 10 ppm, which is the current threshold limit value, the rodents developed minor lesions from exposure.

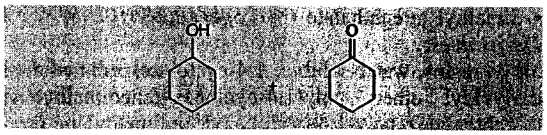
Exposure Limits
TLV-TWA 10 ppm (~18 mg/m³) (ACGIH, MSHA, and OSHA); IDLH 2000 ppm (NIOSH).

Fire and Explosion Hazard
Flammable gas; the gas (vapor) is heavier than air and can travel a considerable distance to a source of ignition and flash back; autoignition temperature 402°C (755°F); fire-extinguishing procedure: shut off the flow of gas; use dry chemical, CO_2, or "alcohol" foam to extinguish fire involving its solution; use a water spray for diluting and flushing the spill and to keep fire-exposed containers cool.

Dimethylamine forms explosive mixtures with air in the range between 2.8 and 14.4% by volume in air. Its reactions with strong acids and oxidizers can be vigorous; contact with acrolein may catalyze violent exothermic polymerization. Dimethylamine may react violently with chlorine and hypochlorites. Explosive reaction may occur in contact with mercury.

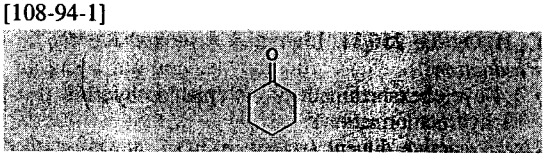
in water: 36 g/l water (20°C). Miscible with oxygenated and chlorinated solvents.
Production:
• cyclohexanol-cyclohexanone, mixed (separation)
• phenol (hydrogenation)
Derivatives:
adipic acid; cyclohexanone; cyclohexene; cyclohexyl acrylate; cyclohexyl chloride; cyclohexyl epoxystearate; cyclohexyl methacrylate; dicyclohexyl phthalate; 2,2′-methylenebis(4-methyl-6-cyclohexylphenol)
Uses: solvent (resins, printing inks)

cyclohexanol-cyclohexanone, mixed
KA oil; ketone-alcohol oil



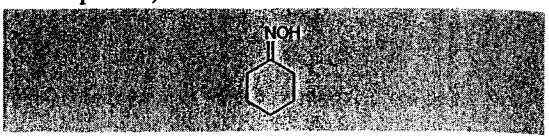
Intermediate stream. Not a commercially traded product.
Production:
• cyclohexane (oxidation)
Derivatives: adipic acid; cyclohexanol; cyclohexanone

cyclohexanone
[108-94-1]



$C_6H_{10}O_1$. M: 98.15. Colourless liquid. BP: 150–158°C. FP: -31°C. d: 0.95 kg/l (20°C). Solubility in water: 23 g/l (20°C). Miscible with most organic solvents. Flash point: 44°C (TCC).
Production:
• cyclohexanol-cyclohexanone, mixed (alcohol oxidation)
• cyclohexanol (alcohol oxidation)
Derivatives:
caprolactone; ciclacillin; cyclobarbital; cyclohexane peroxide; cyclohexanone oxime; cyclohexanone resin; cyclohexylamine; 1,1-di(*t*-amylperoxy)cyclohexane; 1,1-di-(*t*-butylperoxy)cyclohexane; dicyclohexylamine; ethynyl cyclohexanol; hexobarbital; 1-hydroxycyclohexyl phenyl ketone; 1-(4-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline; pyrogallol
Uses: solvent (resins, lacquers, printing inks)

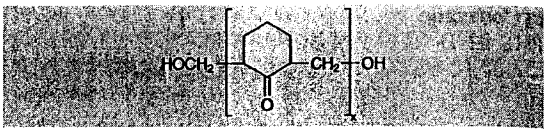
cyclohexanone oxime
[100-64-1]
$C_6H_{11}N_1O_1$. M: 113.17. Solid. MP: 90°C. BP: 206–210°C. Soluble in water and oxygenated solvents.
Production:
• cyclohexanone + hydroxylamine sulphate (oxime formation)
• cyclohexane + nitrosyl chloride (photonitrosation)
• cyclohexanone + hydroxylamine phosphate (DSM HPO process)



Derivatives: caprolactam
Uses: antioxidant

cyclohexanone peroxide *See:* cyclohexane peroxide

cyclohexanone resin
ketone resin



Colourless or pale yellow solid. Acid value: 0 mg KOH/g. Hydroxyl value: 0 mg KOH/g. Soluble in oxygenated solvents. Insoluble in aliphatic solvents and water.
Production:
• cyclohexanone + formaldehyde (carbonyl condensation)
Uses: adhesion promotion agent (printing inks); clear metal cellulose lacquer modifier; tackifier (polyamide hot melt adhesives)

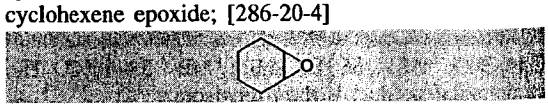
cyclohexene
[110-83-8]

C_6H_{10}. M: 82.15.
Production:
• cyclohexanol (dehydration)
Derivatives:
cyclohexene oxide; cyclohexyl mercaptan; L-lysine

4-cyclohexene-1,2-dicarboximide
See: tetrahydrophthalimide

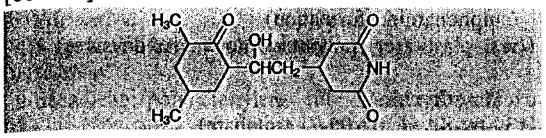
4-cyclohexene-1,2-dicarboxylic acid
See: tetrahydrophthalic anhydride

cyclohexene oxide
cyclohexene epoxide; [286-20-4]



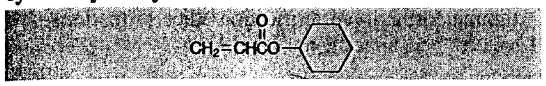
$C_6H_{10}O_1$. M: 98.15. Liquid. BP: 130°C. MP: -30°C.
Production:
• cyclohexene (hypochlorination/dehydrochlorination)
Derivatives: propargite

cycloheximide
[66-81-9]



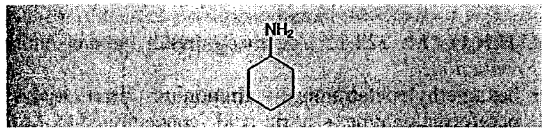
$C_{15}H_{23}N_1O_4$. M: 281.35.
Production:
• microbial fermentation medium + *Streptomyces griseus* bacteria (fermentation/extraction; byproduct of streptomycin production)
Uses:
fungicide/plant growth regulator

cyclohexyl acrylate



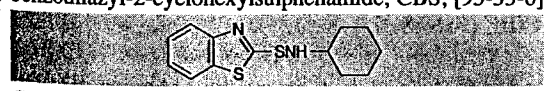
$C_9H_{14}O_2$. M: 154.21.
Production:
• cyclohexanol + acrylic acid (esterification)
Uses: acrylic resin comonomer

cyclohexylamine
CHA; [108-91-8]



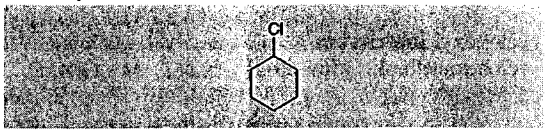
$C_6H_{13}N_1$. M: 99.18. Liquid with a strong, amine odour. BP: 133–134°C. FP: -17°C. d: 0.87 kg/l (4°C). Miscible with water and most organic solvents. Flash point: 28°C (CC).
Production:
• aniline (reduction; coproduced with dicyclohexylamine)
• cyclohexanone + ammonia (reductive ammoniation; coproduced with dicyclohexylamine)
Derivatives:
Acid Blue 62; calcium cyclamate; *N*-cyclohexyl-2-benzothiazolesulphenamide; cyclohexyl isocyanate; *N*-cyclohexylmaleimide; *N*-cyclohexyl-*p*-toluenesulphonamide; dicyclohexylcarbodiimide; *N,N*-diethylcyclohexylamine; *N,N*-dimethylcyclohexylamine; *N*-ethylcyclohexylamine; *N*-methylcyclohexylamine; sodium cyclamate
Uses: corrosion inhibitor (boiler water); process solvent

***N*-cyclohexyl-2-benzothiazolesulphenamide**
benzothiazyl-2-cyclohexylsulphenamide; CBS; [95-33-0]



$C_{13}H_{16}N_2S_2$. M: 264.41. Off-white powder. MP: 95–100°C. d: 1.27 kg/l. Insoluble in water. Soluble in aromatic solvents.
Production:
• 2-mercaptobenzothiazole + cyclohexylamine (oxidative coupling)
Uses:
vulcanisation accelerator

cyclohexyl chloride
chlorocyclohexane; [542-18-7]



$C_6H_{11}Cl_1$. M: 118.61. Liquid. BP: 141–143°C. MP: -44°C. d: 1.00 kg/l (20°C). Insoluble in water. Miscible with oxygenated and aromatic solvents.
Production:
• cyclohexanol (chlorination)
Derivatives:
azocyclotin; cyclomethycaine; cyhexatin

cyclohexyldiethylamine
See: N,N-diethylcyclohexylamine

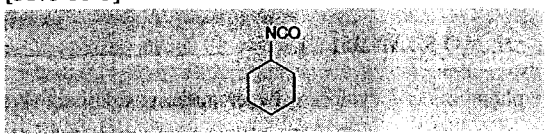
1,4-cyclohexylene glycol
See: 1,4-cyclohexanedimethanol

cyclohexyl epoxystearate

$C_{24}H_{44}O_3$. M: 380.61.
Production:
• oleic acid + peracetic acid + cyclohexanol (epoxidation/esterification)
Uses:
polyvinyl chloride costabiliser/plasticiser

cyclohexylethylamine *See: N*-ethylcyclohexylamine

cyclohexyl isocyanate
[3173-53-3]



$C_7H_{11}N_1O_1$. M: 125.18.
Production:
• cyclohexylamine + phosgene (phosgenation)
Derivatives: glibenclamide; glipizide; hexazinone; hexythiazox; lenacil

***N*-cyclohexylmaleimide**
$C_{10}H_{13}N_1O_2$. M: 179.22.
Production:
• maleic acid + cyclohexylamine (amide formation)

TOXICOLOGICAL ASPECTS OF CYCLAMATE AND CYCLOHEXYLAMINE

Authors: **Barbara A. Bopp**
Robert C. Sonders
Drug Metabolism Department
James W. Kesterson
Pharmaceutical Development Division
Abbott Laboratories
North Chicago, Illinois

CRC Critical Reviews in Toxicology

Referee: A. G. Renwick
Department of Clinical Pharmacology
University of Southampton
Southampton, England

I. INTRODUCTION

Sodium cyclamate, or sodium cyclohexylsulfamate, was synthesized in 1937 by Audrieth- and Sveda[1,2] who accidently discovered its sweet taste. Further studies showed that cyclamate is at least 30 times as sweet as sucrose, is synergistic with saccharin, but does not have the bitter aftertaste characteristic of saccharin.[3,4] Cyclamate was not marketed until 1951 when it was approved by the U.S. Food and Drug Administration (FDA) as a new drug, which was recommended for use as a table-top sweetener by diabetics and others who had to restrict their use of sugar.[5] After enactment of the Food Additive Amendment in 1958, cyclamate was classified by the FDA as a GRAS, or generally recognized as safe, substance.[6] Subsequently, the use of a 10:1 mixture of cyclamate and saccharin[7] in foods and soft drinks became popular and led to a marked increase in the consumption of the artificial sweeteners during the 1960s. Prompted by growing concerns about the safety of this greater intake of cyclamate, additional studies were conducted. It was discovered that cyclamate, which had been thought to be eliminated from the body as the unchanged compound, could be metabolized to cyclohexylamine.[8] Then in 1969, the results of a toxicity study with the 10:1 cyclamate-saccharin mixture were interpreted by the FDA to implicate cyclamate as a bladder carcinogen in rats.[9] Cyclamate was immediately removed from the GRAS list,[10] and in 1970 was banned from use in all foods and drugs.[11] However, many foreign regulatory agencies did not act as precipitously as the FDA, and the use of cyclamate continued in some countries.

In the next few years, many additional toxicity and carcinogenicity studies were conducted with cyclamate, the cyclamate-saccharin mixture, and cyclohexylamine. Based on the results of these studies performed by independent investigators throughout the world, Abbott Laboratories in 1973 filed a food additive petition seeking reapproval for the use of cyclamate as a sweetening agent.[12-14] During the lengthy review process, the FDA requested the National Cancer Institute to convene a panel of scientists to evaluate all the carcinogenicity studies with cyclamate. Their report was published in 1976 and concluded that . . . "[T]he present evidence does not establish the carcinogenicity of cyclamate or its principal metabolite, cyclohexylamine, in experimental animals."[15] In spite of this conclusion, the petition was denied.[16] At the request of Abbott Laboratories, administrative hearings were held in 1977.[17,18] The judge ruled against cyclamate,[19] and finally in 1980, the commissioner of the FDA upheld the denial of the petition.[20]

Prompted by additional studies and criticism of this decision by scientific organizations,[21,22] the Calorie Control Council and Abbott Laboratories filed another food additive petition for cyclamate in 1982.[23] The Cancer Assessment Committee of the Center for Food Safety and

Applied Nutrition at the FDA completed their evaluation of the carcinogenicity bioassays with cyclamate,[24] and at the request of the FDA, a National Academy of Sciences-National Research Council (NAS-NRC) committee has also reviewed the issue of cyclamate carcinogenicity.[25] Action on the 1982 petition is still pending. In contrast to the situation in the U.S., the World Health Organization's Joint Expert Committee on Food Additives has approved the use of cyclamate since 1977,[26] and it is estimated that cyclamate is now available either as a table-top sweetener, or for use in foods and beverages, or both in over 40 countries.[23,27,28]

The purpose of this article is to review the many carcinogenicity, general toxicity, and metabolism studies conducted with cyclamate and cyclohexylamine. With any compound that has generated as much controversy as cyclamate, it is difficult to separate the scientific and "political" issues. However, we will attempt to emphasize the scientific aspects and to critically evaluate the toxicological questions that have been raised about cyclamate and cyclohexylamine.

II. ACUTE TOXICITY

Determinations of the LD_{50}s for sodium cyclamate in mice and rats ranged from 10 to 17 g/kg after oral administration, 6 to 12 g/kg after intraperitoneal administration, and 3 to 5 g/kg after intravenous administration (Table 1). Calcium cyclamate was considerably more toxic especially after parenteral administration, but this difference was probably due to the greater toxicity of the calcium ion. The acute oral toxicity of the 10:1 sodium cyclamate-sodium saccharin mixture was comparable to that of either compound alone, with LD_{50}s ranging from 6 to 21 g/kg in mice and rats. Chronic administration of this mixture of artificial sweeteners had little effect on the LD_{50}. However, the LD_{50} of the cyclamate-saccharin mixture was somewhat lower in newborn rats than adult animals.

Cyclohexylamine is considerably more toxic than cyclamate. The LD_{50}s of intraperitoneally administered cyclohexylamine in mice ranged from 300 to 770 mg/kg and varied with the environmental temperature and whether the animals were isolated or aggregated.[37,38] Lomonova[43] found that the absolute lethal dose (LD_{100}) of orally administered cyclohexylamine base in rats was 500 mg/kg while the maximal tolerated dose (LD_0) was 150 mg/kg. Determinations of the oral LD_{50} of cyclohexylamine in rats ranged from 157 to 614 mg/kg. Chronic administration of a sodium cyclamate-sodium saccharin mixture (10:1) did not affect the acute toxicity of cyclohexylamine.[33,34] As will subsequently be seen, rats and mice tolerated doses in the range of the acute oral LD_{50}s when cyclohexylamine was incorporated into the food during subchronic and chronic toxicity studies. Presumably, the considerably lower toxicity in the feeding studies results from the gradual consumption of cyclohexylamine, a compound that is readily absorbed and rapidly eliminated from the body.

III. PATHOPHYSIOLOGICAL EFFECTS

A. Cyclamate

1. Introduction

During the past 30 to 40 years, a great many subchronic and chronic toxicity studies have been conducted with cyclamate or the cyclamate/saccharin mixture in laboratory animals.[29,31-34,44-73] These studies have generally revealed very few pathophysiological effects associated with the administration of cyclamate, even in very high doses. Considering the large number of studies that have been performed and the great concern over the potential toxicity of cyclamate that arose during the late 1960s, it is not surprising that adverse effects were occasionally reported and frequently attracted much attention. Many of these reports were subsequently shown to be isolated findings that could not be replicated and hence

Table 1
ACUTE TOXICITY OF CYCLAMATE AND CYCLOHEXYLAMINE

Compound	Species	Route	LD_{50}	Ref.
Sodium cyclamate	Mouse	p.o.	10—12 g/kg	29
			11 g/kg	30
			17 g/kg	31
			15.3 g/kg	30
		i.p.	10—12 g/kg	30
			7.1 g/kg	31
		i.v.	4 g/kg	29
			4.8 g/kg	31
	Rat	p.o.	12 g/kg	29
			17.5 g/kg	31
			15.3 g/kg	31
		i.p.	6 g/kg	31
		i.v.	3.5 g/kg	31
	Hamster	p.o.	10—12 g/kg[a]	32
Calcium cyclamate	Mouse	p.o.	7.2 g/kg	30
		i.v.	0.57 g/kg	30
	Rat	s.c.	0.1 g/kg	30
	Hamster	p.o.	4.6 g/kg[a]	32
	Rabbit	i.v.	0.12 g/kg	30
Sodium Cyclamate-Sodium Saccharin (10:1 mixture)	Mouse	p.o.	12.8 g/kg	31
			21.5 g/kg	30
		i.p.	4.6 g/kg	31
	Rat	p.o.	16.5 g/kg	31
			21.5 g/kg	31
	(Progeny of controls)		6.4 g/kg	33, 34
	(Progeny of C/S[b] rats)		7.8 g/kg	33, 34
	(Newborn)		3.3 g/kg	35
		i.p.	6.5 g/kg	31
Cyclohexylamine	Mouse	i.p.	619 mg/kg[e]	36
	(Isolated at 20°C)		770 mg/kg[c]	37, 38
	(Aggregated at 20°C)		520 mg/kg[e]	37, 38
	(Isolated at 28°C)		465 mg/kg[e]	37, 38
	(Aggregated at 28°C)		300 mg/kg[e]	37, 38
		s.c.	1150 mg/kg[d]	39
	Rat	p.o.	614 mg/kg[c,d]	40
	(F)		237 mg/kg[f]	30
	(M)		348 mg/kg[f]	30
	(F)		237 mg/kg[d]	41
	(M)		278 mg/kg[d]	41
	(F)		156 mg/kg[d]	41
	(Pregnant F)		180 mg/kg[d]	41
	(Progeny of controls)		157 mg/kg[d]	33, 34
	(Progeny of C/S rats)		185 mg/kg[d]	33, 34
		i.p.	200 mg/kg[d]	42
	Rabbit	i.v.	150—175 mg/kg[f]	30

[a] 8 days treatment.
[b] C/S = cyclamate-saccharin mixture.
[c] 0.71 mℓ/kg; density = 0.865 g/mℓ.
[d] Cyclohexylamine administered as base.
[e] Cyclohexylamine administered as HCl.
[f] Form of cyclohexylamine not known.

cannot be rightfully attributed to cyclamate. Rather than reviewing each of the many studies with cyclamate, the following section will attempt to evaluate the reported effects of cyclamate on the various organ systems in both animals and man, by presenting positive findings and comparing these with similar studies in which the reported effects were not found. In many cases the findings were not substantiated, and no explanation is possible for the atypical results.

2. Liver

The question of cyclamate-induced liver toxicity was raised in the studies by Gottinger, Hagmuller et al.,[57-58] in which male guinea pigs were given 0.5 or 2% sodium cyclamate in the drinking water. The fluid intake of the control and low dose group was restricted to a level equivalent to that of the high dose group. Mortality among the cyclamate-treated animals was high, but the survival of the controls was also poor, presumably due to the enforced lack of water. Elevations of serum glutamic-pyruvic transaminase and lactic dehydrogenase were observed in the 2% cyclamate group, and histopathological changes in the liver included cellular necrosis and glycogen accumulation in the cytoplasm.

Other studies have, however, failed to demonstrate any similar changes in liver structure or function. Blood chemistry tests indicative of hepatic function have been included in many subchronic and chronic studies with cyclamate or the cyclamate-saccharin mixture in rats (0.5 to 5% in the diet),[33-34,59,63] dogs (0.5 to 1.5 g/kg/day),[29,31,46-47,62] and monkeys (200 mg/kg/day)[48-49] and have not revealed any abnormalities. Similarly, cyclamate has not caused any histopathological changes in the liver of mice (5 to 7% in the diet),[45,59,60] rats (1 to 5% in the diet),[29,31,33-34,53-54,59,64-65,71-73] dogs (0.5 to 1.5 g/kg/day)[29,31,46-47,62] or monkeys (200 mg/kg/day).[48-49]

Stein et al.[74] reported a mild vesiculation of the endoplasmic reticulum associated with vacuolization of the liver cells in monkeys given a single oral 4 or 8 g/kg dose of sodium cyclamate. However, electron microscopic examination of the livers from monkeys given sodium cyclamate, either as a single, 4 to 7 g/kg dose,[75] or as daily 200 mg/kg doses for 8 years,[48-49] could not confirm these findings and revealed no ultrastructural changes attributable to the sweetener.

Clinical studies have also failed to demonstrate any adverse effect on liver function or morphology. Blood chemistry tests indicative of liver function and bromosulphthalein retention tests were not affected by the administration of cyclamate in daily doses of 2 to 10 g to healthy volunteers, diabetics, or patients with liver and kidney diseases.[76-83] Liver biopsies obtained from diabetics ingesting cyclamate in doses of about 40 to 800 mg/day revealed no evidence of triglyceride accumulation, glycogen deposition, or any histopathological changes attributable to the artificial sweetener.[84]

3. Kidney

The kidney may be adversely affected by high doses of cyclamate in rats.[33-34,52,54,59,64-6] A slightly increased incidence of nephritis and nephrosis has been reported in some chroni studies, but these changes were relatively common in the control rats as well.[33-34,] Th most frequent changes attributable to cyclamate involved calcification in the kidneys, whic was sometimes accompanied by hyperplasia of the renal epithelium.[33-34,54,59,64-65] These effects were best described by Friedman et al.[54] In one of their studies, Osborne-Mende rats were fed a chow diet containing 0.4, 2, or 10% sodium or calcium cyclamate for 8 to 101 weeks. Nephrocalcinosis, typified by calcium deposits in the interstitium of th collecting tubules, renal pyramids or calyx, was observed in 5% of the control rats and 4 to 49% of the rats receiving cyclamate. The incidence of nephrocalcinosis was dose related with most of the cases occurring in the rats given 10% cyclamate, but was similar with th sodium and calcium salts. Calyceal polyposis, defined as edematous, hemorrhagic, o myxomatous polypoidal formation on the calyces, occurred in 53 and 44% of the ra

ingesting the sodium and calcium salts, respectively. The mechanism involved in the renal calcification is unknown, but X-ray examinations of the skeleton and teeth of these rats provided no evidence of any generalized disruption of calcium metabolism.

Nephrocalcinosis and renal hyperplasia are by no means universal findings in rats receiving cyclamate. No adverse effects on the kidney were seen in most studies with lower doses of cyclamate (≤2% in the diet) or shorter treatment times,[29,31,63,73] and even some chronic studies in which rats were given 5% cyclamate in the diet have not reported any renal pathology attributable to the sweetener.[53,68,71-72] Urinalysis results from rats receiving cyclamate have generally been unremarkable[29,33-34,63] except for occasional findings of increased levels of salts, including oxalates, urates, and phosphates in one study[69] and calcium, phosphorus, and magnesium in another.[85] Clinical chemistry tests indicative of renal function were also unaffected by the administration of cyclamate to rats.[33-34,59,63]

Renal calcification has generally not been observed in other species. Three chronic feeding studies in mice given up to 5 to 7% sodium cyclamate in the diet have failed to demonstrate any histopathological changes in the kidneys which were attributable to the sweetener.[45,59-60] Similarly, urinalysis, renal function tests, and microscopic examination of the kidneys from dogs given cyclamate or the cyclamate-saccharin mixture (0.5 to 1.5 g/kg/day) have not revealed any adverse effects.[29,31,46-47,62] Also, Coulston et al.[48-49] did not observe any histopathological changes in the kidneys of monkeys given sodium cyclamate (200 mg/kg/day) for 8 years.

Clinical studies have indicated that the administration of cyclamate in doses of 2 to 10 g/day does not affect renal function in man.[76-83,86] Zöllner et al.[78-80] gave patients with chronic kidney or liver diseases daily doses of sodium cyclamate (2 or 5 g) for up to 3 years. Careful monitoring of the blood chemistry tests and urinalysis results gave no indication of any adverse effect on the renal function of the patients. Van der Hem et al.[86] obtained similar results in renal-impaired patients who were given calcium cyclamate in daily doses of 5.3 g for 6 months.

4. Gastrointestinal Tract

Softening of the feces and diarrhea are probably the most consistently observed effects in animals and man receiving cyclamate. Rats fed diets containing 5 to 10% cyclamate often developed soft, loose stools or even a watery diarrhea; with lower concentrations, around 1 to 2%, the fecal pellets were formed, but were larger and had a higher water content.[31,33-34,53-54,64-65,68,71-72,87] These effects tended to be intermittent and frequently were most pronounced during the first few weeks of cyclamate administration.[64-65,68] The tendency of high dietary cyclamate concentrations to cause diarrhea in rats was exacerbated when the artificial sweetener was administered in certain semisynthetic or purified diets.[88-92]

Diarrhea also occurred in dogs given high doses of sodium cyclamate (2 to 4 g/kg/day).[29] Semisolid stools were observed in dogs during the first two weeks of treatment with 1.5 g/kg/day doses of sodium cyclamate, but the consistency of the feces subsequently returned to normal; lower doses, 150 to 500 mg/kg/day, were generally without effect.[62] In monkeys, 2 g/kg/day doses of sodium cyclamate caused softening of the feces and occasional rectal prolapse.[30]

In clinical studies, the administration of cyclamate in doses of about 5 g per day frequently led to soft heavy stools, and as the dose was increased to 10 to 16 g per day, diarrhea developed in many, but not all of the subjects.[76,81-83,93-95] However, the doses used in these clinical safety studies were very high, and such levels were only rarely attained when cyclamate was widely used in artificially sweetened foods and beverages. On a body weight basis, children did not appear to be any more sensitive to the laxative effects of cyclamate than adults.[76,96]

Hwang[87] demonstrated that the laxative action of cyclamate was related to its osmotic activity and was similar to the effect exerted by sodium sulfate. In rats, both cyclamate and

sulfate increased the intestinal motility, the liquidity of the stools, and the amount of fluid retained in the intestinal lumen. These effects were significantly correlated with the osmotic activity of the unabsorbed fraction of the salts, and after correction for absorption and the degree of ionization, there were no differences in the relative potencies of sodium cyclamate, calcium cyclamate, and sodium sulfate. No systemic effects that could contribute to the laxative activity of cyclamate were seen with parenteral administration or in isolated intestinal preparations.

Most subchronic and chronic toxicity studies have not demonstrated any pathological abnormalities in the gastrointestinal tract following the administration of cyclamate. Bernier et al.[97] did, however, observe some changes in the intestines of male Wistar rats fed diets containing 5% calcium cyclamate for 4 months. The feces of these rats became soft shortly after the animals started to ingest cyclamate and remained that way throughout the study. At necropsy, the small intestine was moderately distended, and the cecum was markedly expanded and filled with fluid. Histologically, edema and clubbing of the villi were observed in about 80% of the animals. These occurred throughout the intestine, but were especially pronounced in the ileum. The observed changes were consistent with the increased movement of water through the intestinal wall, due to the osmotic activity of cyclamate.

5. Heart

Extremely high doses of calcium cyclamate induced myocardial calcification and sclerosis of the coronary vessels in Syrian golden hamsters.[98-100] In one such study,[98-99] hamsters were given 0.2 g of calcium cyclamate, orally, two or three times a day for 6 days, corresponding to a total dose of about 4 to 6 g/kg/day. All of the animals developed focal calcified lesions in the myocardium, which were accompanied by varying degrees of degeneration and necrosis. Monckeberg arteriosclerosis was observed in the coronary arteries of 65% of the animals. In addition, calcification and necrosis of the skeletal muscle occurred in 45% and nephrocalcinosis in 70% of the hamsters. Mortality totaled 75% during the 6-day study. Other calcium salts, including the chloride, acetate, aspartate, and ascorbate, did not produce similar lesions, indicating that the calcium ion was not solely responsible for the cardiac lesions.

Weiss et al.[101] further observed that these high doses of calcium cyclamate (2 to 3 g/kg, twice daily) caused diarrhea, weight loss, and EKG changes (increased PR intervals, widening of the QRS complex, depressed ST segments and T wave abnormalities), which are typical of hypokalemia. Equimolar amounts of calcium chloride also caused diarrhea and similar EKG abnormalities in about 20% of the hamsters. However, calcium lactate or acetate, sodium cyclamate (1 to 2 g/kg, twice daily), and lower doses of calcium cyclamate (0.5 to 1 g/kg, twice daily) were tolerated by the animals and did not affect the EKG. The fact that the EKG abnormalities were only observed concomitantly with diarrhea suggested that they were probably secondary to hypokalemia and fluid loss and were not indicative of a direct cardiotoxic effect of cyclamate.

In a lifetime study with calcium and sodium cyclamate (up to 1.25% in the drinking water), Althoff et al.[32] observed that the incidence of vascular calcinosis was higher in the cyclamate-treated hamsters than the contemporary controls, but was not in excess of the incidence generally seen in their colony of animals. In contrast to these findings in hamsters, a species that is frequently prone to calcifying disorders, there is no evidence of any cardiovascular lesions in rats treated with calcium or sodium cyclamate. Friedman et al.[74] specifically stated that no signs of myocardial or vascular calcification were observed in Osborne-Mendel rats fed chow diets containing 0.4, 2, or 10% calcium or sodium cyclamate for 101 weeks or in Holtzman rats fed semisynthetic diets containing 1 or 2% calcium cyclamate for 75 weeks. No treatment-related cardiac lesions were found by Taylor et al.[71-72] in Charles River CD rats given diets containing 5% calcium cyclamate for over 2

years or by Nees and Derse[64-65] in Sprague-Dawley rats given up to 10% calcium cyclamate in the diet for 1 year. Other studies in rats,[29,31,33-34,53,59,68] mice[45,59-60,67] and dogs[29,31,46-47,62] also support the conclusion that cyclamate does not induce myocardial and vascular calcification in these species.

6. Blood

Hematology studies incorporated into many of the subchronic and chronic toxicity studies in mice[60] and rats[29,31,33-34,53,63-65,71-72] given cyclamate or the cyclamate-saccharin mixture have generally not revealed any adverse effects. The only treatment-related effect has been a slight anemia, characterized by reductions in the red blood cell counts and/or hemoglobin concentrations, in mice receiving 7% sodium cyclamate in the diet for 80 weeks[45] and rats given 10% calcium cyclamate in the diet for 1 month.[61] However, the hemoglobin levels were only decreased by 7 to 17%, and these effects only occurred with extremely high doses of cyclamate. Hematological abnormalities have not been observed in dogs[29,31,46-47,62] or monkeys[48-49] given cyclamate or the cyclamate-saccharin mixture. Similarly, numerous clinical studies have indicated that cyclamate administration does not adversely affect the hematology parameters in man.[76-83,86,93]

Gottinger et al.[57] suggested that cyclamate might also interfere with blood coagulation and potentiate the effects of the coumarin anticoagulants in rabbits. Once again, other studies in rats,[33-34,63] dogs,[46-47,62] and man[79-82,102-103] have not confirmed these effects. Prothrombin time was not affected in rats given the cyclamate-saccharin (10:1) mixture (2500 mg/kg/day) for two years.[33-34] Similarly, the prothrombin time, clotting time, partial thromboplastin time, and fibrinogen content were not changed in dogs receiving the same cyclamate-saccharin mixture in doses up to 1.5 g/kg/day for 2 years.[46-47]

Zöllner and Schnelle[79] found that the one-stage prothrombin time and platelet count were unchanged in patients with chronic hepatic and renal diseases who had been given daily doses of sodium cyclamate (5 g) for at least 3 months. Egli[102] gave sodium cyclamate (5 g/day) to 20 volunteers for 4 weeks, and determined many factors involved in the blood coagulation process, including the clotting time, prothrombin (Quick) time, prothrombin, factors V, VII, VIII, and X, antithrombin III, thrombin time, platelet count, and the thrombelastogram. Again, there was no evidence of any adverse effect of cyclamate on blood coagulation. Even higher daily doses of sodium cyclamate, 10 to 16 g, had no effect on the prothrombin time of healthy volunteers in the study by Wills et al.[81,82] Holcenberg et al.[103] found that cyclamate (3 to 4.5 g/day) did not potentiate the anticoagulant effects of warfarin in man, and in vitro studies showed that very high concentrations of cyclamate (>1000 mcg/mℓ) were needed to even slightly displace warfarin from its binding sites in human plasma. For comparison, the plasma levels in man are unlikely to exceed 20 mcg/mℓ, even with large doses of cyclamate (see Section VIII.A.1.).

C. Endocrine Glands

1. Thyroid

Questions about a possible effect of cyclamate on the thyroid gland largely centered on the report of elevated protein bound iodine (PBI) levels in the blood of men receiving sodium cyclamate in doses of up to 10 to 16 g/day.[81-82,104] In spite of the high PBI levels, thyroxine concentrations were not increased, and none of the subjects showed any signs of thyroid hyperactivity. Further investigations indicated that the observed effect on PBI was not attributable to cyclamate, but was caused by the presence of iodine in the erythrosine dye used to color the capsules in which the cyclamate had been administered.[82] When monkeys that had maintained normal PBI levels during 6 months of treatment with sodium cyclamate (200 mg/kg/day) were given a similar dose of this dye, their PBI levels showed a similar increase.

At least two other clinical studies have shown that the total serum iodine and PBI le
els were not elevated in subjects ingesting sodium cyclamate in daily doses of 2 to
g.[76-77,79] Similarly, PBI levels and thyroid function were not affected in rats,[58-59] guinea pigs,
dogs,[46-47,62] or monkeys[82] given cyclamate in chronic or subchronic studies. Furthermor
histopathological changes have not been found in the thyroid gland of mice,[45] rats,[33-34,53,
or dogs[46-47,62] receiving cyclamate.

b. Adrenals

Only a single study has suggested that changes in the adrenals might be associated wi
cyclamate administration. Nees and Derse[64-65] reported a slight increase in the absolute a
especially the relative weights of the adrenals from rats given diets containing 5 or 10
cyclamate for one year. Histologically, the adrenals showed subtle changes in the corte
primarily involving the zona granulosa. However, histopathological changes in the adren
have not been found in numerous other studies with cyclamate or the cyclamate-sacchar
mixture in mice (5 to 7% in the diet),[45,60] rats (5% in the diet),[33-34,53,71-73] or dogs (1.5
kg/day).[46-47,62]

c. Pancreas and Blood Sugar

Hagmuller et al.[58] suggested that cyclamate might interfere with sugar metabolism, bas
on their findings of increased pancreatic alpha cells, the presence of Armanni-Ebstein ce
in the kidney, glycogen deposits in the liver, and potentiation of the hypoglycemic eff
of tolbutamide in guinea pigs given 0.5 to 2% sodium cyclamate in the drinking wat
However, other studies provided no evidence of an adverse effect of cyclamate
the structure of the pancreas in a variety of species, including mice,[45,60] rats,[31,33-34,53
dogs.[29,46-47,62] Usami et al.[105] showed that cyclamate did not affect the arginine-indu
secretion of insulin and glucagon in an isolated perfused rat pancreas model. Furthermo
the blood glucose levels were not affected either by the acute subcutaneous injection
sodium cyclamate in rats (100 to 200 mg/kg)[29,106] or by the daily oral administration
cyclamate or the cyclamate-saccharin mixture in rats,[33-34,59,63] dogs,[46-47,62] or monkeys;[
Studies investigating a possible interaction of cyclamate with oral hypoglycemic agent
animals have reported both potentiation and diminution of the blood glucose changes;[5
However, the purported effects were based on relatively small changes that would have li
clinical significance.

Any questions about the possible effect of cyclamate on blood glucose or an inte
tion between cyclamate and hypoglycemic drugs are best answered by the studies in
Several clinical studies have clearly demonstrated that cyclamate ingestion does not
nificantly affect the blood sugar levels in healthy persons[76-77,81-82,107] or diabetics.[76,108-11
et al.[76,108-109] closely followed 30 diabetics during a 13-month period while the in
Several clinical studies have clearly demonstrated that cyclamate ingestion does not
nificantly affect the blood sugar levels in healthy persons[76-77,81-82,107] or diabetics.[76,108-11
et al.[108-109] closely followed 30 diabetics during a 13-month period while the in
cyclamate-containing food was encouraged and then cyclamate consumption was
mented by the daily administration of 2 g of the cyclamate-saccharin mixture. The
included some requiring insulin, some taking sulfonylurea drugs, and some control
diet alone. Blood glucose levels were not affected by the increasing doses of cyclam
and cyclamate consumption did not affect the individual requirements for insulin
sulfonylurea drugs. Pröls et al.[110] administered 5 daily doses of sodium cyclamate
diabetics and also saw no changes in the blood or urinary glucose levels in the
taking sulfonylurea drugs, insulin, or no drugs. Thus, cyclamate does not appear to
affect the blood glucose levels of diabetics or their requirements for hypoglycemic

8. Reproductive System

a. Male

Testicular atrophy has been reported in several chronic studies with cyclamate in rats (Table 2). Unfortunately the testicular effects were not carefully described in many of these studies, thus making any evaluation of the results more difficult. The "testicular atrophy" was defined by reductions in the absolute and/or relative weight of the testes in some studies, by macroscopic changes observed at necropsy in other studies, and by histological changes in yet other studies.

Ferrando and Huchet[52] reported testicular atrophy in four of six second generation rats treated with 3% sodium cyclamate in the diet. The testicular changes were accompanied by "weight loss", but more extensive conclusions were precluded by insufficient data. Nees and Derse[64-65] fed rats diets containing 5 or 10% calcium cyclamate for 1 year. Body weight gain was depressed by cyclamate in both the rats fed *ad libitum* and those on a limited feeding regimen. The absolute and relative testicular weights were not reduced, but the incidence and severity of testicular atrophy were greater in the cyclamate treated rats than the controls. However, these investigators commented that the changes noted in the rats receiving cyclamate may have been "an aggravation of a normal aging process".

In a chronic toxicity and reproduction study conducted by Oser et al.,[33-34] groups of 35 Wistar-derived male rats were given a sodium cyclamate-sodium saccharin (10:1) mixture at doses of 500, 1120, and 2500 mg/kg/day for 24 months. After 78 weeks, cyclohexylamine was added to the diets of about half of the rats to provide doses of 25, 56, and 125 mg/kg, corresponding to 10% conversion of the cyclamate dose. A dose-related reduction in weight gain was observed, and at the end of the study the body weight of the high dose males was only about two thirds of the controls. Testicular weights were not determined, but based on gross or histological examinations, testicular atrophy occurred in 3 control rats, no rats at 500 mg/kg/day, 1 rat at 1120 mg/kg/day, and 11 rats at 2500 mg/kg/day. The incidence of testicular atrophy in the high dose group was about the same in the rats receiving the diets supplemented with cyclohexylamine and those given only the cyclamate-saccharin mixture. Oser et al.[33] questioned the significance of these testicular changes, since no impairment in the fertility of the males had been seen in the earlier reproduction studies and the rats had become quite old.

In a study by Taylor et al.,[71-72] Sprague-Dawley rats were fed a diet containing 5% calcium cyclamate for their lifetime. Absolute testicular weights were not decreased in the rats sacrificed at 14 or 18 months, but by the end of the study (about 28 months) the weights of the testes and several other organs were significantly reduced. Since the body weights of these rats were also decreased, the relative testicular weights of the cyclamate-treated rats were not markedly lower than the controls. The incidence of macroscopic testicular atrophy was greater in the rats receiving cyclamate than the controls near the end of the study (18 months to termination), but only one of the cyclamate-treated rats had been affected before 18 months. Thus, the testicular effects appeared to develop only after the rats had become quite old and apparently lost a substantial amount of weight.

The most pronounced testicular effects were reported in the study of Ikeda and his colleagues,[55-56] in which Wistar-derived rats were fed diets containing 5% sodium cyclamate or 5% of a sodium cyclamate-sodium saccharin mixture (10:1) for up to 28 months. The body weights in the cyclamate and cyclamate-saccharin groups were consistently decreased, but the severity of the changes increased from a 17% reduction at 12 months to 45 to 49% at 24 to 28 months. The absolute weights of the testes were significantly decreased in the rats receiving the artificial sweeteners at both 12 and 24 months, but not at 28 months. The lower weights were not restricted to the testes, but were seen in the other organs as well. The incidence of macroscopic and histological testicular atrophy was considerably greater in the rats receiving cyclamate or the cyclamate-saccharin mixture than the controls, and also increased as the duration of the study was extended.

Table 2
TESTICULAR EFFECTS IN RATS GIVEN CYCLAMATE

Study	Duration	Treatment	Body wt (g)	Testes weight		Testicular atrophy	
				Absolute (g)	Relative	Gross	Histo.
Ferrando and Huchet[52]	18—24 months	Control	—	—	—	—	0/6
		Na Cyclamate-0.8%	—	—	—	—	0/5
		1.6%	—	—	—	—	0/6
		3.0%	"Weight loss"[b]	—	—	—	4/6
Ikeda et al.[55-56]	12 months	Control	498	2.9	0.53	1/13	1/13
		Na Cyclamate-5%	411*	2.4*	0.57	3/16	7/15
		Cyclamate-Saccharin-5%	415*	2.1*	0.51	4/10	5/10
	24 months	Control	664	3.4	0.52	1/11	2/11
		Na Cyclamate-5%	342*	1.5*	0.42	6/11	11/11
		Cyclamate-Saccharin-5%	364*	1.4*	0.37	9/11	10/10
	28 months	Control	596	2.4	0.39	0/3	1/3
		Na Cyclamate-5%	303*	2.7	0.87	4/6	6/6
		Cyclamate-Saccharin-5%	335*	1.6	0.49	7/8	7/7
	Dying	Control	—	—	—	6/27	—
		Na Cyclamate-5%	—	—	—	12/23	—
		Cyclamate-Saccharin-5%	—	—	—	14/25	—
Nees and Derse[64-65]	12 months	Control-ad lib	520	3.1	0.61		
		Ca Cyclamate-5%	438	3.2	0.70		
		Ca Cyclamate-10%	414	3.5	0.85		
	12 months	Control-restricted feeding	359	3.7	1.0		
		Ca Cyalamte-5%	314	3.3	1.1		
		Ca Cyclamate-10%	296	3.4	1.2		
Oser et al.[33-34]	24 months	Control	608	—	—	—	3/35[a]
		Na Cyclamate-1.0%	549	—	—	—	0/35[a]
		Na Cyclamate-2.2%	487	—	—	—	1/35[a]
		Na Cyclamate-5.0%	407	—	—	—	11/35[a]

		Control	538	3.8	0.72	—	—
		Ca Cyclamate-5%	559	3.8	0.70	—	—
		Control	672	3.8	0.58	0	—
		Ca Cyclamate-5%	598	3.8	0.68	1/11	—
	Termination	Control	588	3.8	0.65	2/29	—
		Ca Cyclamate-5%	440*	2.6*	0.58	10/32	—

Note: * $p < 0.05$.

[a] Initial group size = 35.
[b] Body weight data not available.

The testicular atrophy observed in the cyclamate treated rats in these studies appeared (1) to occur only with high doses of cyclamate (e.g., 5 to 10% in the diet or about 2.5 tc 5.0 g/kg/day); (2) to be accompanied by reductions in the weights of other organs and the body weight, so that the relative testicular weights were often not decreased; and (3) tc become more evident as the rats grew old and had received cyclamate for long periods o time. These trends all suggested that the testicular changes seen with cyclamate were probably not indicative of a direct toxic action on the testes, since that type of effect usually manifest itself after a relatively short period of treatment.[14,113] It is well known that malnutrition and certain dietary deficiencies can cause hypoplasia of the testes and adversely affect sper matogenesis in laboratory animals.[114-115] Nutritional deficiencies could have resulted from the decreased consumption of the diets containing high levels of the sweetener or from impaired absorption associated with the laxative effects of high cyclamate concentra tions.[14,113] Also, degenerative changes in the testes frequently occur spontaneously in olde rats. For example, James and Heywood[116] found atrophy of the germinal epithelium in 19% of their colony of 2-year-old male Sprague-Dawley rats, whereas Goodman et al.[117] note testicular atrophy in 30% of their 2-year-old Osborne-Mendel rats. Incidences as high a 50% have occasionally been reported.[118] Hence, the testicular atrophy seen in the rat receiving cyclamate appears to be more typical of a secondary or indirect effect, possibly resulting from decreased body weight, nutritional deficiencies, the aging process, or combination of these factors.

The possibility that cyclohexylamine may be responsible for, or contribute to, the testicula atrophy seen in the rats receiving cyclamate must also be considered. The study most likely to be influenced by cyclohexylamine is that of Oser et al.,[33-34] since the metabolite wa added to the treatment regimen near the end of the study. However, the similar incidenc of testicular atrophy in the rats given and not given cyclohexylamine would argue against a significant role of the metabolite. It also seems unlikely that the conversion of cyclamat to cyclohexylamine could have been responsible for the effects seen in the other studies. I 10% of the cyclamate were converted to cyclohexylamine, the resultant dose from the 5% dietary level would only be 125 mg/kg. Based on the feeding studies with cyclohexylamine that dose would probably not cause an appreciable incidence of testicular atrophy. It is als unlikely that many rats would consistently convert cyclamate to cyclohexylamine at a leve as high as 10%, since the average conversion by the rats in Oser's study was only aroun 1 to 4%.[33]

It must be pointed out that testicular atrophy did not occur in all of the rat feedin studies with cyclamate. No adverse effects were evident in the two lower dose groups fro Oser's[33-34] study (500 and 1120 mg/kg/day) with the cyclamate-saccharin mixture. This consistent with the lack of any gross or microscopic changes in the testes of rats given 0. to 2.0% of the cyclamate-saccharin mixture in the diet for 6 months[31] or 1% cyclamate the diet for about 2 years.[29] Furthermore, Friedman et al.[54] stated that no gross testicul lesions were apparent in Osborne-Mendel rats given 0.4, 2, or 10% sodium or calciu cyclamate in the diet for 88 to 101 weeks. Schmähl[68] also conducted thorough gro examinations of rats given 2 or 5% sodium cyclamate or the cyclamate-saccharin mixtu in the diet for their lifetime and made no mention of any testicular atrophy. Although the studies did not include histological examinations of the testes, the testicular changes we evident at necropsy in the studies by Oser,[33-34] Taylor,[71-72] and Ikeda,[55-56] suggesting th histological examination was not essential.

Testicular atrophy has not been reported with cyclamate in any species other than the r Treatment of mice with up to 5 to 7% sodium cyclamate in the diet did not cause any adver effects on the testes.[45,60] Sodium cyclamate and the cyclamate-saccharin mixture (10:1) ha been given orally to dogs in doses up to 1.5 g/kg/day for 3 months[62] or 2 years respectively, without affecting the testes. Two chronic studies in monkeys also gave

indication of adverse testicular effects. Coulston et al.[48-49] treated rhesus monkeys with sodium cyclamate orally in a dose of 200 mg/kg/day, 6 days a week. The testes of one animal sacrificed after 91 months of treatment were examined microscopically, and no deviations from the normal morphology were detected. Sieber and Adamson[70] have given monkeys 100 or 500 mg/kg/day doses of sodium cyclamate orally, 5 days a week, for over 12 years. No differences were found between the control and cyclamate-treated monkeys with respect to testicular size, testicular morphology, endocrine status, semen count, or sperm morphology.[119] Analysis of urine samples collected from these males indicated that most of the monkeys were converting small amounts of cyclamate to cyclohexylamine, and two were metabolizing a large percentage (13 to 37%) of the dose.[119] The lack of any adverse testicular effects in this study is especially significant since these monkeys had been receiving relatively large doses of cyclamate for an extended period of time and were converting cyclamate to cyclohexylamine in amounts comparable to those found in a group of human subjects.

b. Female

There is no evidence of any adverse effect from cyclamate treatment on the female reproductive organs. Vaginal smears performed on rats receiving up to 5% of the cyclamate-saccharin mixture in their diets revealed no alterations in the estrous cycle.[33-34] Furthermore, no histopathological changes have been found in the ovaries or uterus of mice,[45,60] rats,[29,31,33-34,53] and dogs[46-47,62] given cyclamate or the cyclamate-saccharin mixture.

B. Cyclohexylamine

1. Introduction

After cyclohexylamine was identified as the major metabolite of cyclamate, its toxicity became a significant concern. Since little information was available in the literature, chronic studies were initiated in rats[120] and dogs,[121] but the doses (15 mg/kg/day) subsequently proved to be low. At least two subchronic[122-123] and three chronic[68,124-126] studies have now been conducted with much higher doses of cyclohexylamine in rats, while two chronic studies have been performed in mice,[60,127] and one in dogs.[121] These studies will be briefly reviewed, and then two areas of major toxicological concern, the effects of cyclohexylamine on the cardiovascular system and the testes, will be discussed in detail.

In the two 3-month studies, rats were given diets containing cyclohexylamine hydrochloride at concentrations ranging from 0.01 to 2.5%[122] and from 0.06 to 0.6%.[123] All animals receiving 2.5% cyclohexylamine hydrochloride died within 5 days, and intestinal hemorrhages were seen at necropsy. No deaths occurred at the lower doses. The hematology, serum chemistry, and urinalysis parameters were generally unaffected by cyclohexylamine. In one study, a slight decrease was seen in the renal concentrating ability of the females given 0.6%. Body weight gain was decreased at dietary levels down to 0.1 to 0.2%, but food intake was also reduced, presumably due to the bitter taste of the diets containing cyclohexylamine. However, paired-feeding studies showed that the reduced food intake at 0.6 and 1% did not entirely account for the body weight decrements, thus suggesting a direct effect of cyclohexylamine at these high levels. Absorption of nutrients appeared to be normal, but there was a slight increase in the oxygen consumption of the cyclohexylamine treated (0.6%) rats. Many organ weights were lower in the rats given 0.2% cyclohexylamine and above, but these changes were generally related to the decreased body weight of the animals. The only pathological changes involved the testes.

In the three chronic studies, rats were given diets containing cyclohexylamine hydrochloride at concentrations of (1) 0.06, 0.2, and 0.6%[124]; (2) 0.4%;[68] or (3) in varying concentrations to provide daily doses of 15, 50, 100, or 150 mg/kg/day[125-126] (150 mg/kg/day = 0.3% in the diet). The results were generally similar to those in the 3-month studies.

Body weight gain was decreased at 0.2% in the diet or 100 mg/kg/day and above, but
growth reductions were associated with decreased food and water intake. Other chan
attributable to the lower body weights resulting from decreased consumption of the unp
atable diets included decreased absolute organ weights, decreased serum urea concentratio
increased serum albumin levels, and reduced incidences of tumors and many histopatl
logical changes. Besides the testicular effects which will be discussed later, the only chan
possibly related to treatment in the study by Gaunt et al.[124] were a slight anemia, a fail
to produce concentrated urine, and an increase in the number of macrophages in the alv
of the lungs of the rats given 0.6% cyclohexylamine in the diet. The only histopathologi
changes possibly attributable to cyclohexylamine in the study by Oser et al.[125-126] w
slightly increased incidences of mucosal thickening of the bladder wall, renal calcificati
and testicular atrophy.

Cyclohexylamine appeared to be somewhat less toxic in mice. In one of the two chr
studies, mice were given diets containing cyclohexylamine hydrochloride at concentrati
of 0.03, 0.1, and 0.3% for 80 weeks.[127] The highest level corresponded to about a 400
kg/day dose of the hydrochloride or 300 mg base/kg/day. Survival, body weight gain, f
consumption, water intake, hematology parameters, major organ weights, and tumor i
dence were not affected by cyclohexylamine. The only histopathological change possi
related to treatment was an increased incidence of minor hepatic changes (cell vacuoliza
or polyploidy) in the females at 0.3%, but since a similar effect was not seen in the ma
its significance is questionable.

In a six-generation study,[60] mice were given diets containing 0.5% cyclohexylan
sulfate; long-term (21 months) studies were conducted in three generations while the o
generations were followed for 4 months. Body weight gain was depressed, particularl
the females, but survival was increased in the cyclohexylamine groups. Hematology
aminations did not reveal any changes attributable to treatment, and the histopatholo
findings were similar in the control and experimental groups.

A chronic study was also performed in groups of six beagle dogs which were g
cyclohexylamine sulfate in daily oral doses of 0, 0.15, 1.5, and 15 mg/kg/day.[121] C
hexylamine did not affect growth, behavior, hematology, serum chemistry, urinalysi
hepatic and renal function tests. One male and one female from each group were sacri
after 1 year of treatment, and the organ weight data and histological examinations o
tissues did not reveal any abnormalities attributable to cyclohexylamine. After about 4 y
the doses were increased to 50, 100, and 150 mg/kg/day. The animals lost weight aft
dosage increase, but subsequently slowly regained the weight. Clinical pathology tes
not affected by the higher doses, and no histopathological changes attributable to
hexylamine were seen in the animals that died during the study or those sacrificed
end of the 9.5 year period.

2. Sympathomimetic Activity

a. Cardiovascular Effects

Even as early as 1910, Barger and Dale[128] described the pressor activity of
hexylamine, but with the discovery that cyclamate was metabolized to cyclohex
more interest developed in the sympathomimetic effects of this amine. In anes
cats or dogs, intravenous administration of cyclohexylamine caused hypertension
chronotropic and ionotropic effects, and peripheral vasoconstriction.[129-1
hexylamine did not impair the blood pressure responses to norepinephrine, epine
acetylcholine, histamine, isoproterenol, or dimethylphenylpiperazinium,[131,136-139] alth
slight potentiation of the effects of norepinephrine and epinephrine has been obs
especially in reserpinized animals.[130-132] Neither spinal section, ganglionic bloc
adrenalectomy influenced the activity of cyclohexylamine.[36,129-132,136-137] How

pressor effects of cyclohexylamine were blocked by phenoxybenzamine, phentolamine and tolazoline, indicating the involvement of α-receptors,[36,129,132,135-141] while the cardiac effects were inhibited by propranolol and other β-blockers.[129,132,135,140-141] In most cases, the effects of cyclohexylamine were diminished by pretreatment with cocaine, guanethidine and reserpine, but they could be partially restored in reserpinized animals by the infusion of norepinephrine.[36,129-132,135-142] Tachyphylaxis was observed with repeated doses of cyclohexylamine both in vivo and in vitro, and again the effects could be partially restored by the administration of norepinephrine.[36,129,131-132,136-137,143] Cyclohexylamine also caused a dose-dependent inhibition of ^{3}H-norepinephrine uptake and decreased the endogenous norepinephrine levels in the rat heart.[134,144] Thus, the above evidence indicates that cyclohexylamine is primarily an indirectly acting sympathomimetic agent, similar to tyramine, but it is probably 100 to 1000 times less potent than tyramine.[141]

Cyclohexylamine produced similar cardiovascular effects after oral and intravenous administration to anesthetized animals, but was considerably less potent when given orally.[130,134] Classen estimated that the minimal effective doses in cats were 0.05 mg/kg intravenously and 10 to 15 mg/kg orally.[134] The marked difference in potency between the two routes of administration is surprising since cyclohexylamine is rapidly and completely absorbed from the gastrointestinal tract, but it may be related to the peak plasma levels, rather than the total area under the plasma concentration-time curve.

Orally administered cyclohexylamine also increases blood pressure in unanesthetized animals, but in place of the positive chronotropic effects, a reflex bradycardia usually occurs. The cardiovascular effects of a single oral dose of cyclohexylamine have been studied most thoroughly in man. In healthy volunteers, the mean arterial blood pressure increased about 30 mm of Hg at 1 hr after a 10 mg/kg dose.[145-146] A somewhat smaller, but still statistically significant, rise in blood pressure was seen with a 5 mg/kg dose, and no significant changes occurred after a 2.5 mg/kg dose. A slight decrease in the heart rate accompanied the vasopressor effects of the two high doses. The cyclohexylamine levels in plasma were closely correlated with the increases in the mean arterial blood pressure, and it was estimated that the lowest level of cyclohexylamine to cause a significant hypertensive effect was about 0.7 to 0.8 mcg/mℓ.

In contrast to the acute effects of orally administered cyclohexylamine, most chronic studies in animals have failed to demonstrate any significant cardiovascular effects. Hypertension did not occur in two feeding studies with cyclohexylamine in rats, even at doses considerably above those that might be expected to increase blood pressure. In a study by Collings and Kirkby,[122] rats fed diets containing 0.01 to 1.0% cyclohexylamine hydrochloride for 90 days did not show an elevation in blood pressure, and the pressor response to norepinephrine was not affected in these animals. Schmähl[68] directed special attention toward the cardiovascular system in his 2 year study with 0.4% cyclohexylamine in the diet (approximately 200 mg/kg/day). The blood pressure of these rats was not increased, and no histopathological changes were seen in the heart or vascular system. It is unlikely that a species difference is responsible for the lack of any demonstrable increase in the blood pressure of rats since Classen et al.[129] reported that the acute administration of cyclohexylamine caused similar cardiovascular effects in cats, rats, and guinea pigs. Hence, the lack of a pressor effect is probably due either to the lower circulating levels of cyclohexylamine attained with the gradual consumption of cyclohexylamine containing diets or possibly to the development of tolerance to the effects of the amine.

The lack of a hypertensive effect in the feeding studies is particularly significant since they represent an experimental model that more closely reflects the gradual conversion of cyclamate to cyclohexylamine in the lower gastrointestinal tract. The latter situation would probably give a relatively constant rate of cyclohexylamine formation and absorption throughout the day. Using the pharmacokinetic parameters reported for cyclohexylamine in man[146] and assuming zero-order absorption over a 24-hr period, it can be estimated that the maximal

plasma levels achieved from a 5 mg/kg dose of cyclohexylamine would be below the low concentration (0.7 to 0.8 mcg/mℓ) associated with a hypertensive response. Even with a mg/kg dose, the expected maximal levels in plasma would only be slightly above 1 m mℓ. Assuming that 25% of the ingested cyclamate is converted to cyclohexylamine, an mg/kg dose of sodium cyclamate, or 5.6 g for a 70 kg man, would be needed to gener a 10 mg/kg dose of cyclohexylamine.* Thus, based on this analysis, it is not surprising t hypertensive effects have not been associated with the use of cyclamate.

Numerous animal and clinical studies would support the contention that the poten vasopressor effects of cyclohexylamine do not pose a real hazard to cyclamate users. I pertension did not develop either in rats given diets containing up to 5% sodium cyclam for 2 years[68] or in dogs given up to 1 to 1.5 g/kg/day doses orally for 1 to 3 months.[62] Likewise, clinical studies have repeatedly failed to detect an increase in the blood press of subjects ingesting relatively large doses of cyclamate,[76-82,108-109] but most of these stuc were completed prior to the discovery that cyclamate was converted to cyclohexylamine few studies have attempted to correlate the blood pressure responses and cyclohexylam excretion. Litchfield and Swan[148] monitored the blood pressure and heart rate of five clohexylamine excretors who were given 5 g of sodium cyclamate for 7 to 8 days, but s no changes that were attributable to the artificial sweetener. Unfortunately, all of th subjects proved to be relatively poor converters. Periodic blood pressure measurements h also been made in other subjects who were among the highest known converters. Colling gave four converters and three nonconverters daily sodium cyclamate doses of up to 1(25 mg/kg/day for 18 days without noting any change in blood pressure or heart rate; maxi conversion in these subjects ranged from 4 to 44%. Sonders and Wiegand[150] also failec detect any increase in the blood pressure of one converter who received 3 g of sodi cyclamate daily for 14 days; maximal conversion in this subject was 41%. Hence, available evidence suggests that, although cyclohexylamine is an indirectly acting symp omimetic agent and has the inherent ability to increase blood pressure, these effects are realized when the amine is formed in vivo from orally administered cyclamate.

b. Cardiopathy

Classen and his colleagues[135,151-154] found that cyclohexylamine can aggravate the car necrosis induced by epinephrine in rats sensitized with 9-α-fluorocortisol acetate. Nei cyclohexylamine (5 mg per 100 g rat, sc) nor epinephrine (300 mcg) alone induced vis lesions in the heart, and when given together only 1 in 10 rats developed necrotic foci rats pretreated with 9-α-fluorocortisol acetate, a 5 mg dose of cyclohexylamine failec induce the lesions, and, even with a dose that killed 40% of the animals, only 10% sho signs of cardiac necrosis. In contrast, epinephrine (75 to 600 mcg) caused dose-depen myocardial necrosis in the rats previously sensitized with the steroid. The subcutane administration of cyclohexylamine along with both epinephrine and fluorocortisol did, h ever, increase the frequency and severity of the cardiac necrosis, as well as the resul mortality.

Similar types of pathological changes were seen in the hearts of the rats pretreated fluorocortisol and epinephrine, either with or without cyclohexylamine.[151] The heart bec enlarged and necrotic foci of various sizes were scattered through the myocardium. In more advanced lesions, the cytoplasm of the myocytes contained lipid droplets and calc granules. Edema and an inflammatory reaction were also observed. In the affected ar the smaller vessels were dilated and occasionally partly necrotic, but occlusive thrombi v not observed in the vessels. The cardiopathy induced by epinephrine in rats previo

* If 100% of a 80 mg/kg dose of sodium cyclamate is converted to cyclohexylamine, the resultant do cyclohexylamine is about 40 mg/kg due to the difference in molecular weights (201/99). Hence, if only of the cyclamate is converted to cyclohexylamine, the resultant dose of cyclohexylamine would be 10 m

sensitized by steroid treatment is presumably dependent on the circulating levels of the catecholamine.[151] Hence, it has been suggested that cyclohexylamine might aggravate this toxicity by elevating the catecholamine levels, either through a release of endogenous amines or by inhibiting their reuptake.

c. Other

Cyclohexylamine can also exert other sympathomimetic effects, including contraction of the nictitating membrane in anesthetized cats,[129,135,138-139] contraction of the rat vas deferens preparation and potentiation of its response to norepinephrine,[155-156] and inhibition of glucose or tolbutamide-mediated insulin secretion in an in vitro hamster pancreas preparation.[157] The effects on both the nictitating membrane and vas deferens were inhibited by α-blockers.[129,139,155]

In contrast to the ability of epinephrine to elevate blood glucose levels, there is little evidence to suggest that cyclohexylamine exerts a similar effect.[146,154,158] Classen et al.[154] observed that the acute subcutaneous administration of cyclohexylamine had no effect on the blood glucose, free fatty acid (FFA), or potassium levels of rats and did not modify the hyperglycemia, hyperkalemia, or increase in FFA elicited by epinephrine. Furthermore, the blood sugar levels of rats and dogs were not affected by the ingestion of high doses of cyclohexylamine in several subchronic or chronic toxicity studies.[121,123-126] Gondry,[159-160] however, did observe that the blood sugar levels from a test dose of glucose (1 g/kg, i.v.) returned to normal slightly more slowly in rats maintained on diets containing very high concentrations of cyclohexylamine (1%) for 1 to 3 months. The insulin levels of these rats were not affected.

In one of the few human studies with cyclohexylamine, Eichelbaum et al.[146] found that the blood glucose and serum potassium levels of adult males were not significantly changed by single oral 2.5, 5, or 10 mg/kg doses, although the FFA concentrations were slightly elevated with the highest dose. The failure of cyclohexylamine to increase the glucose levels is consistent with the extensive clinical data demonstrating that the blood sugar levels of healthy adults or diabetics are not affected by the ingestion of cyclamate.[76-77,81-82,108-112]

3. Testes

The organ that is clearly the most sensitive to any chronic toxic effect of cyclohexylamine is the testes. As previously discussed, cyclamate may also affect the testes, but the effects elicited by the two compounds are quite different.[113] With cyclamate, the testicular effects in rats appear to be secondary to nutritional deficiencies combined with the aging process. In contrast, cyclohexylamine has a direct toxic effect on the rat testes that cannot be accounted for by body weight changes and is readily demonstrable in subchronic studies. The testicular effects of cyclohexylamine in rats were initially defined in three 90-day studies in which the hydrochloride salt was incorporated into the food at concentrations ranging from 0.01 to 1.0%.[122,123,161] Subsequently, the effects were studied in more detail in animals fed diets providing a constant mg/kg dose of cyclohexylamine.[162] Because of the importance of this effect and since much of this material is unpublished, each study will be discussed individually in some detail.

In the first study by Collings and Kirkby[122] at Unilever, groups of 15 or 16 male rats were given diets containing cyclohexylamine hydrochloride at concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, or 1.0% for 90 days. Body weight gain and food intake were significantly decreased at dietary levels of 0.2% and above (Table 3). The absolute testicular weights were depressed at the two highest concentrations, while the relative weight was increased at 0.5%, but decreased at 1%. Degeneration of the tubular epithelium was seen in both testes of 13 out of 15 rats given 1% cyclohexylamine hydrochloride, with ≥95% of the tubules being affected in 8 rats, ≥70% in 4 rats, and ≥40% in 1 rat. The incidence of other histopathological changes in the testes (i.e., reduced spermatogenesis, intertubular edema,

International Chemical Safety Cards

CYCLOHEXYLAMINE

ICSC: 0245

CYCLOHEXYLAMINE
Cyclohexanamine
Aminocyclohexane
Aminohexahydrobenzene
$C_6H_{11}NH_2$
Molecular mass: 99.2

CAS # 108-91-8
RTECS # GX0700000
ICSC # 0245
UN # 2357
EC # 612-050-00-8

HAZARD SYMBOLS
Consult National Legislation

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/ SYMPTOMS	PREVENTION	FIRST AID/ FIRE FIGHTING
FIRE	Flammable.	NO open flames, NO sparks, and NO smoking. NO contact with oxidants.	Powder, alcohol-resistant foam, water in large amounts, carbon dioxide.
EXPLOSION	Above 26°C explosive vapour/air mixtures may be formed.	Above 26°C closed system, ventilation, and explosion-proof electrical equipment.	In case of fire: keep drums, etc., cool by spraying with water.
EXPOSURE		AVOID ALL CONTACT!	IN ALL CASES CONSULT A DOCTOR!
INHALATION	Corrosive. Burning sensation. Cough. Laboured breathing.	Ventilation, local exhaust, or breathing protection.	Fresh air, rest. Half-upright position. Refer for medical attention.
SKIN	Corrosive. Redness. Pain. Blisters.	Protective gloves. Protective clothing.	Remove contaminated clothes. Rinse skin with plenty of water or shower. Refer for medical attention.
EYES	Corrosive. Redness. Pain. Severe deep burns.	Face shield.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
INGESTION	Corrosive. Abdominal cramps. Burning sensation. Vomiting. Collapse.	Do not eat, drink, or smoke during work.	Rinse mouth. Refer for medical attention.

SPILLAGE DISPOSAL	STORAGE	PACKAGING & LABELLING
Collect leaking liquid in sealable containers. Absorb remaining liquid in sand or inert absorbent and remove to safe place (extra personal protection: self-contained breathing apparatus).	Fireproof. Separated from strong oxidants, acids, food and feedstuffs.	Do not transport with food and feedstuffs. C symbol R: 10-21/22-34 S: 36/37/39 UN Haz Class: 8 UN Subsidiary Risks: 3 UN Pack Group: II

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ICSC: 0245

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CYCLOHEXYLAMINE

ICSC: 0245

IMPORTANT DATA

PHYSICAL STATE; APPEARANCE:
COLOURLESS LIQUID, WITH PUNGENT ODOUR.

PHYSICAL DANGERS:
The vapour is heavier than air.

CHEMICAL DANGERS:
The substance decomposes on heating producing toxic gases (nitrogen oxides). The substance is a strong base, it reacts violently with acid and is corrosive. Reacts violently with strong oxidants causing fire hazard.

OCCUPATIONAL EXPOSURE LIMITS:
TLV: 10 ppm; 41 mg/m^3 (as TWA) (ACGIH 1992-1993).
PDK: 1 mg/m^3 (USSR 1993).

ROUTES OF EXPOSURE:
The substance can be absorbed into the body by inhalation and by ingestion.

INHALATION RISK:
A harmful contamination of the air can be reached rather quickly on evaporation of this substance at 20°C.

EFFECTS OF SHORT-TERM EXPOSURE:
Corrosive. The substance is corrosive to the eyes, the skin and the respiratory tract. Inhalation of the vapour may cause lung oedema (see Notes). The effects may be delayed.

EFFECTS OF LONG-TERM OR REPEATED EXPOSURE:
Repeated or prolonged contact may cause skin sensitization.

PHYSICAL PROPERTIES

Boiling point: 134°C
Melting point: -18°C
Relative density (water = 1): 0.9
Solubility in water: good
Vapour pressure, kPa at 20°C: 1.2
Relative vapour density (air = 1): 3.4
Relative density of the vapour/air-mixture at 20°C (air = 1): 1.03
Flash point: (c.c.) 26°C
Auto-ignition temperature: 293°C
Explosive limits, vol% in air: 1.5%-9.4%

ENVIRONMENTAL DATA

NOTES

The symptoms of lung oedema often do not become manifest until a few hours have passed and they are aggravated by physical effort. Rest and medical observation are therefore essential. Immediate administration of an appropriate spray, by a doctor or a person authorized by him/her, should be considered.

Transport Emergency Card: TEC (R)-71
NFPA Code: H 2; F 3; R 0

ADDITIONAL INFORMATION

ICSC: 0245 **CYCLOHEXYLAMINE**

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from the same rats used in their bone marrow work and observed a dose-related increase in the percentage of spermatogonial cells with chromosome breaks. Again, there was no evidence of any chromosome exchanges or translocations attributable to cyclohexylamine. These animals had been given five daily intraperitoneal injections of cyclohexylamine in doses of 1 to 50 mg/kg. Ford et al.[356] attempted to replicate Legator's work using the 50 mg/kg dose, but failed to obtain an increased incidence of chromosome abnormalities in the spermatogonial cells. Similarly, Oser et al.[126,340] did not observe any chromosome aberrations in the testes of rats maintained on diets providing cyclohexylamine at doses of 50 to 150 mg/kg/day. Negative results with spermatogonia were also reported by Machemer and Lorke,[357] who treated Chinese hamsters orally with cyclohexylamine (100 mg/kg/day) for five days, and by Kaziwara and Mizutani,[177] who gave mice a single intraperitoneal injection of cyclohexylamine (40 to 80 mg/kg). Using the indirect technique of examining spermatocytes for damage induced in the spermatogonia, Cattanach and Pollard[358] also failed to detect any adverse effects from cyclohexylamine treatment in mice (50 or 100 mg/kg/day for 5 days, i.p.). Therefore, the only evidence that cyclohexylamine can induce genetic damage in male germ cells is that of Legator et al.[338] Their findings were based entirely on chromosome breaks and could not be confirmed by other studies in rats, Chinese hamsters, or mice. Hence, it seems unlikely that cyclohexylamine causes heritable genetic damage in mammalian germ cells.

F. Dominant Lethal Tests

An extensive group of dominant lethal tests performed by Lorke and Machemer[359-361] clearly demonstrated that cyclamate does not induce dominant lethal mutations in mice (Table 19). In the first study,[359] male mice were given 10 g/kg/day doses of sodium cyclamate orally for 5 days and then mated with untreated females each week for 10 weeks. In the second study,[360] both male and female mice were fed diets containing 1% sodium cyclamate (about 2 g/kg/day) for 10 weeks and a single mating trial was conducted. Neither treatment adversely affected the fertility of the animals, and pre- and postimplantation losses were not increased in either study. The final approach involved treating female mice with a single 10 g/kg dose of sodium cyclamate during proestrus and then mating them with untreated males.[361] The timing of the experiment was adjusted so that preovulatory oocytes would be exposed to the test compound. Known mutagens, e.g., cyclophosphamide, were shown to induce dominant lethal mutations in this test, but cyclamate had no effect.

Aeschbacher et al.[230] investigated a possible comutagenic effect of cyclamate in combination with the *N*-methyl nitrosourea that would be formed in the stomach of mice given methyl urea and sodium nitrite. The weak dominant lethal effects seen in this study were solely due to the methylurea plus nitrite treatment and were not enhanced by cyclamate administration. One dominant lethal test has been conducted in rats given semisynthetic diets containing 10 or 20% casein and 1 or 2% calcium cyclamate for 10 months.[54] Fertility was low in all groups, including the controls, but was further decreased in the group receiving 2% cyclamate with the low-protein diet. However, pre- and postimplantation losses were not significantly increased by cyclamate administration. Hence, there is no experimental evidence to suggest that cyclamate causes dominant lethal mutations in either mice or rats.

The results of the dominant lethal tests with cyclohexylamine are not as uniformly negative as those with cyclamate. Two early studies by Peterson et al.[363-364] suggested that cyclohexylamine might induce dominant lethal mutations in male mice which were given five daily intraperitoneal injections of cyclohexylamine at a dose of 100 mg/kg/day. During both the 3 and 6 week mating trials, the postimplantation losses were significantly greater in the cyclohexylamine-treated mice (15 to 18%) than the controls (4 to 5%). However, the fertility rate was quite low in the 6 week study (32 to 35%), and the average number of implants in the control group was atypically low (4.7) in the 3 week test. Furthermore, the number

Table 19
DOMINANT LETHAL STUDIES WITH CYCLAMATE AND CYCLOHEXYLAMINE

Compound	Dose	Route	Duration	Species	Treated sex	Mating schedule	Results	Ref.
Na Cyclamate	10 g/kg	Po	5 days	Mouse	M	10 weekly matings	–	359
Na Cyclamate	1% (2 g/kg)	Food	10 weeks	Mouse	M + F	Single mating	–	360
Na Cyclamate	10 g/kg	Po	Single	Mouse	F	Single mating	–	361
Ca Cyclamate	132—660 mg/kg	Ip	Single	Mouse	M	8 weekly matings	–	362
	500—1000 mg/kg	Po	5 days	Mouse	M	8 weekly matings	–	
Na Cyclamate	1.9 g/kg	Po	1 week	Mouse	M	1 and 3 weeks	–	230
+ Methylurea	300 mg/kg	Po	1 week					
+ Na Nitrite	15 mg/kg	Po	1 week					
Ca Cyclamate	1—2%	Food	10 months	Rat	M	Single mating	–	54
Cyclohexylamine	100 mg/kg	Ip	5 days	Mouse	M	6 weekly matings	+	363
(as base)	100 mg/kg	Ip	5 days	Mouse	M	3 weekly matings	+	364
Cyclohexylamine ($as SO_4$)	102 mg/kg	Po	5 days	Mouse	M	8 weekly matings	–	365
Cyclohexylamine ($as SO_4$)	0.11% or 136 mg/kg	Food	10 weeks	Mouse	M + F	Single mating	–	360
Cyclohexylamine ($as SO_4$)	102 mg/kg	Po	Single	Mouse	F	Single mating	–	361
Cyclohexylamine	5—25 mg/kg	Ip	Single	Mouse	M	8 weekly matings	–	362
(as base)	13.7—27.3 mg/kg	Po	5 days	Mouse	M	8 weekly matings	–	
Cyclohexylamine (as base)	50—100 mg/kg	Ip	5 days	Mouse	M	3 weekly meetings	–	358
Cyclohexylamine (as base or HCl)	50 mg/kg	Ip	Single	Mouse	M	3 weekly matings	–	356
Cyclohexylamine (as base)	100—300 mg/kg	Ip	1 day	Rat	M	2 weekly matings	Preimplantation loss	327
Cyclohexylamine ($as SO_4$)	150 mg/kg	Po	65 days	Rat	M		Decreased fertility and implantations	188

of live embryos was not reduced with cyclohexylamine or the positive reference compound, in spite of the increased postimplantation loss. Considering these inconsistencies, the significance of Peterson's findings must be questioned.

All other studies failed to demonstrate any dominant lethal mutations in mice receiving cyclohexylamine. Cattanach and Pollard[358] used a dosing regimen (50 or 100 mg/kg/day, i.p., for 5 days) similar to Peterson's and found that the fertility rate as well as the pre- and postimplantation losses were not adversely affected by cyclohexylamine. Negative results were also obtained in two other small studies with cyclohexylamine-treated mice.[356,362] Lorke and Machemer[360,361,365] conducted a series of three dominant lethal tests with cyclohexylamine, similar to those performed with cyclamate. Oral treatment of the male mice (100 mg/kg/day) for 5 days,[365] of both the males and females (~136 mg/kg/day) for 10 weeks,[360] or just the females (100 mg/kg)[361] failed to decrease the fertility rate or increase the pre- and postimplantation losses. Hence, the preponderance of the evidence certainly suggests that cyclohexylamine does not induce dominant lethal mutations in mice.

Two dominant lethal tests have been conducted in rats, and in both cases, cyclohexylamine only increased the preimplantation loss, which may be due to a variety of nongenetic causes. Postimplantation loss, which is generally considered to be the more reliable indicator of true dominant lethal effects, was not increased in either study. Khera and Stoltz[188] treated male rats with cyclohexylamine sulfate (142 to 220 mg/kg/day) orally for 65 days. Fertility and the number of implantation sites were decreased, but there was no increase in the number of resorption sites. In the study by Green et al.,[327] male rats were given 100 or 300 mg/kg doses of cyclohexylamine, administered in two intraperitoneal injections about 4 hr apart. Cyclohexylamine treatment significantly increased the preimplantation loss, but not the number of early deaths. In a subsequent experiment,[327] 35% of the eggs taken from the females mated with cyclohexylamine-treated males failed to divide during the first 48 hr, suggesting that a lack of fertilization may have been responsible for the preimplantation loss. It should also be noted that the high intraperitoneal doses of cyclohexylamine used in this study reached the toxic level, causing significant weight loss and even killing several animals.

Overall, there is very little evidence to suggest that cyclohexylamine induces dominant lethal mutations in either mice or rats. Peterson's positive results in mice could not be confirmed by any other investigator in extensive tests that used a variety of different conditions. The positive findings in rats only involved preimplantation losses, which were most likely attributable to nongenetic causes. Therefore, these studies should not be interpreted as being indicative of a dominant lethal effect from cyclohexylamine treatment.

G. Miscellaneous Other Tests

Cyclamate and cyclohexylamine have been evaluated in several other mutagenicity or carcinogenicity screening tests (Table 20). Of these systems, probably only the cell transformation test has achieved any degree of extensive use. This system, developed by Styles, Purchase, and their colleagues[366-368] at ICI, was found to be more than 90% accurate in predicting the carcinogenic activity of a group of 120 organic chemicals. The test uses two cell lines, baby Syrian hamster kidney cells (BHK 21/C13), and human lung cells (WI-38), which are exposed to the test compound in a liquid tissue culture medium containing a rat liver metabolizing system (S-9). After exposure, cell transformation is assessed by the growth of the cells on a semisolid agar medium. Sodium cyclamate had no effect in two experiments with kidney cells at concentrations up to 2500 and 4000 mcg/mℓ. Cyclohexylamine was also inactive in both the kidney and lung cell lines at concentrations up to 250 mcg/mℓ.

Cyclohexylamine was one of the 120 test compounds used to evaluate the cell transformation test, Ames test, and four other short-term carcinogenicity tests.[368] It gave positive results in the mouse sebaceous gland suppression test,[369] based on a decrease in the ratio of

Table 20
MISCELLANEOUS TESTS WITH CYCLAMATE AND CYCLOHEXYLAMINE

Test	Compound	Concentration	Results	Ref.
Cell transformation (human lung and Syrian hamster kidney cells)	Cyclamate	0.25—4000 mcg/mℓ	–	366—368
	Cyclohexylamine	0.08—250 mcg/mℓ	–	
Mouse sebaceous gland supression	Cyclohexylamine	2.4 mg/mouse	+	369
Degranulation of endoplasmic reticulum from rat liver	Cyclohexylamine	12 mcg/mℓ	+	370
Tetazolium-reduction by mouse skin	Cyclohexylamine	—	–	371
Subcutaneous implant in mouse	Cyclohexylamine	0.02 mMol or 2 mg	–	372
In vitro sister chromatid exchange				
Chinese hamster ovary cells and human lymphocytes	Na Cyclamate	1,000—10,000 mcg/mℓ	+	373
	Cyclohexylamine	10—100 mcg/mℓ		
In vivo sister chromatid exchange				
Chinese hamster bone marrow	Na Cyclamate	10 g/kg, p.o.	–	374
	Cyclohexylamine	—	–	
Mouse spot test	Cyclohexylamine	100—200 mg/kg, i.p.	+ (Variable)	375
Mouse micronucleus test	Ca Cyclamate	300—2500 mg/kg/day 5 days, i.p.	–	294, 295, 376

sebaceous glands to hair follicles following topical application of the test compound, and an in vitro test based on degranulation of the rat liver rough endoplasmic reticulum.[370] In addition to the Ames and cell transformation tests, negative results were obtained in the tetrazolium reduction test[371] and subcutaneous implant test[372] in mice. The former evaluated the reduction of tetrazolium by mouse skin that had been exposed to the test compound in vivo. The latter involved a histological assessment of the tissue that formed around a subcutaneously implanted millipore filter containing the test compound. In contrast to the Ames test and cell transformation test, these four other systems were only about 60 to 70% accurate in predicting the carcinogenic activity of the 120 test compounds.

Wolff[373] used the sister chromatid exchange test, which detects reciprocal exchanges of DNA between two sister chromatids that have been stained differentially by 5-bromodeoxyuridine during two previous cell divisions. A small, dose-related increase in sister chromatid exchanges was seen in Chinese hamster ovary cells or human lymphocytes treated with high concentrations of sodium cyclamate (1,000 to 10,000 mcg/mℓ) and in human lymphocytes exposed to cyclohexylamine (10 to 100 mcg/mℓ). In contrast to these in vitro results, Renner[374] found no evidence of mutagenic effects with cyclamate or cyclohexylamine in an in vivo sister chromatid exchange test using bone marrow cells from Chinese hamsters.

Fahrig[375] concluded that cyclohexylamine was weakly active in the mouse spot test, which detects mutations involving several different recessive coat-color genes. However, the experiment had to be repeated seven times with variable results and the data pooled before this conclusion was reached. Moreover, cyclohexylamine was administered intraperitoneally in doses of 100 or 200 mg/kg on the 10th day of gestation. An intraperitoneal injection is certainly not the preferred route of administration and may be particularly inappropriate for a test involving fetal animals.

Heddle and Bruce[294-295] reported that calcium cyclamate was inactive in the micronucleus test, in which somatic cells are examined for the presence of centromeric chromosome fragments (micronuclei). Female mice were given five daily intraperitoneal injections of calcium cyclamate in doses ranging from 300 to 2500 mg/kg/day, and newly formed polychromatic erythrocytes from the femoral bone marrow were used for the assay.

H. Summary

The mutagenic potential of cyclamate and cyclohexylamine has been thoroughly evaluated in a battery of test systems. It is, of course, necessary to employ a variety of tests to determine the mutagenic potential of any compound, since different endpoints, including both gene mutations and chromosomal changes, must be assessed. Recently, much progress has been made toward standardizing the test procedures and criteria for establishing positive effects. However, many of the tests with cyclamate were done in the 1960s and 1970s when the methodology was still evolving. This does not necessarily invalidate the results of these early studies, but any evaluation of the data must include a careful examination of the techniques and the endpoints used for assessing mutagenicity, in addition to the experimental results.

Cyclamate and cyclohexylamine were clearly not mutagenic in the Ames test and other bacterial tests. Cyclohexylamine was also inactive in Drosophila, but the results with cyclamate in this assay system are to date inconclusive. The most conflicting results have been obtained in the in vitro and in vivo cytogenetic studies with somatic cells where both positive and negative studies abound. The test systems employed a wide range of cells including bone marrow, peripheral leukocytes, fibroblasts, and kidney cells, taken from even a wider range of animal species, including rat, Chinese hamster, kangaroo rat, lambs, gerbil, rabbit, and man. The positive results were restricted to an increased incidence of gaps and/or breaks, which are generally considered to be nonspecific lesions that may be subjected to spontaneous repair and are not necessarily indicative of direct genetic damage. There is no conclusive

evidence for a treatment-related increase in the incidence of exchange
cations, which are the more reliable indicators of genetic damage. Mor
findings occurred more frequently in the in vitro studies which often u
far in excess of those that might be achieved in vivo. Indeed, the c
conducted in humans receiving high doses of cyclamate, many of wl
cyclohexylamine, provide no conclusive evidence of a significant mu
particular importance in assessing the mutagenic potential of any compo
with mammalian germ cells. The cytogenetic studies in germ cells of
and the dominant lethal tests are convincingly negative and support t
cyclamate and cyclohexylamine do not induce heritable genetic damage

When the entire battery of mutagenicity tests with cyclamate and
evaluated, the evidence suggests that neither compound represents a sig
hazard. Similar conclusions have also been reached in reviews of the
and Lorke,[357] Cattanach,[298] and Cooper,[377] as well as the Food Additive
Committee of Great Britain.[225] The NAS-NRC committee[25] also evaluat
tests as indicators of carcinogenicity and concluded that there was little li
cyclamate or cyclohexylamine was a DNA-reactive carcinogen. Howe
recommended that assays for mammalian cell DNA damage, mammalian
tests, and more definitive cytogenetic studies be conducted to complete
data base for the two compounds.

VII. HUMAN TOXICITY

In the 1950s and 1960s, many clinicial studies were performed to
cyclamate and the cyclamate-saccharin mixture in man. Specific aspec
which are summarized in Table 21, have previously been mentioned in
this review. It is, however, worth emphasizing the fact that these studie
to demonstrate any clinically significant effects associated with the adm
mate, except for a laxative action at extremely high doses. The doses w
to 5 g range, but were increased to 10 g or more in several studies. Subje
adults and children, as well as diabetics and patients with gastrointestin
diseases. Extensive laboratory tests and physical examinations were perf
effects associated with the administration of cyclamate.

In addition to these specially designed studies, cyclamate has had a re
of safe use in drugs, foods, and beverages. Aside from occasional cas
dermatitis thought to be of an allergic nature,[378-384] there are very few re
effects associated with the ingestion of cyclamate. Moreover, the few ac
were reported in the literature (renal tubular acidosis,[384-385] birth defect
cancer[262]) were largely based on circumstantial evidence and could not be
to the cyclamate.

VIII. DISPOSITION*

A. Cyclamate

1. Absorption, Distribution, and Excretion

The absorption of orally administered cyclamate has been determin
excretion data in a variety of species. Several investigators have reporte
one third of orally administered cyclamate was absorbed in rats.[87,391-39
been studied much less extensively, but dogs[396] appear to absorb about 4
dose, rhesus monkeys, and guinea pigs about two thirds of the dose,[391,3

* The disposition of cyclamate has previously been reviewed in References 388 to 39(

Table 21
MAJOR CLINICAL SAFETY STUDIES WITH CYCLAMATE OR CYCLAMATE-SACCHARIN

Compound	Subjects	No	G/Day	Duration	Findings	Ref.
Cyclamate-saccharin	Healthy adults	30	1.8—6.4	12 months	No significant changes in laboratory tests, hepatic and renal function tests, PBI, blood pressure, or physical examinations; transient diarrhea in five subjects	77
Ca Cyclamate saccharin	Healthy adults	32	1.5—7	6 weeks	No effect on gastrointestinal motility, but increased stool weight and softness at doses over 7 g	94
		28	7—12	2 weeks		
Ca Cyclamate	Healthy adults	6	5	7.5 months	No significant changes in laboratory tests or physical examinations; stool softening, but no increase in bowel movements	83
Na Cyclamate	Healthy adults	2	5	2.5 weeks	Electrolyte and nitrogen balance not impaired	111
Na Cyclamate	Healthy adults	8	5	1 week	No significant findings in laboratory tests, blood sugar, or EKG; soft stools and diarrhea at 5 g	111
	Adult patients	16	2.5	1 week		
	Adult diabetics	4	0.6	7 months		
Na Cyclamate	Healthy adults	5	5	1 week	Stools soft; slight increase increase in urine volume	95
Na Cyclamate	Healthy adults	17	2	Single dose	No effects on blood sugar, pulse rate or blood pressure	107
Ca Cyclamate	Healthy children and adolescents	184	1.0—1.5 g/30 lb	3—6 months	Stools soft; no significant changes in laboratory tests and physical examinations	96
Na Cyclamate-saccharin	Adult patients with gastrointestinal disorders	20	4—5	6—10 weeks	No significant effects in laboratory tests; less constipation	93
Cyclamate-saccharin	Adult diabetics	34	1.1 (avg)	6 months	No significant changes in laboratory tests, physical status, or requirements for hypoglycemic drugs	108, 109
			3.2 (avg)	6 months		
Ca Cyclamate	Older patients with renal impairment	15	5.3	6 months	No change in renal function as reflected by laboratory tests and physical examination	86
Na Cyclamate	Healthy adults	16	5	1 week	No changes in blood pressure, heart rate, or EKG	148
Na Cyclamate	Healthy adults	20	5	4 week	No effect on blood coagulation	102
Na Cyclamate	Adult diabetics	30	3	5 days	No effect on blood sugar or urinary excretion of glucose	110

Table 21 (continued)
MAJOR CLINICAL SAFETY STUDIES WITH CYCLAMATE OR CYCLAMATE-SACCHARIN

Compound	Subjects	No	G/Day	Duration	Findings	Ref.
Cyclamate	Adult diabetics	≥60	0.04—0.8	2 weeks—4 years	Liver biopsies showed no effects on triglycerides or glycogen and no histopathological changes	84
Na Cyclamate	Adult patients with hepatic or renal diseases	38	2—5 g	1—3 years	No significant changes in laboratory tests, hepatic or renal function tests, PBI or blood coagulation	78—80
Na Cyclamate	Healthy adults	24	5, 10 or 16 g (16 g dose decreased to 3 g in some subjects)	1—7 months	No significant effects on laboratory tests, hepatic and renal function tests, blood pressure, sperm in semen; effects on PBI due to dye in capsules; softening of stools or diarrhea at doses of 10 to 16 g, but not 5 g	81, 82

From Berryman, G. H., Hazel, G. R., Taylor, J. D., Sanders, P., and Weinberg, M., *Am. J. Clin. Nutr.*, 21, 673, 1968. With permission.

90% of the dose.[391,398] The data reported for the absorption of cyclamate in man correlate better with the results in rats or dogs than monkeys, guinea pigs, or rabbits. Schoenberger et al.[83] administered cyclamate orally to six human subjects at a dose of 5 g/day for 7.5 months and reported that an average of 37% of the daily intake was recovered in the urine. Sonders et al.[150,399,400] gave cyclamate to 150 adults and 49 children and also recovered about 37% of the ingested dose in the urine as unchanged cyclamate. The urinary excretion appeared to be similar in the children who drank a cola containing cyclamate and the adults who ingested capsules of cyclamate.

Following oral administration of ^{14}C-cyclamate to two human volunteers at a dose of 5 g, the cyclamate levels in the plasma reached a peak of 20 mcg/mℓ by 6 to 8 hr and then declined with an apparent half-life of 8 hr.[337,401-402] This half-life in man agreed well with those based on the excretion data from rats (6.6 hr) and dogs (8.8 hr).[396] However, urinary excretion appeared to be considerably faster after parenteral administration of cyclamate,[83,393,398] suggesting that the half-life found after oral administration may be influenced by the slow absorption of cyclamate from the gastrointestinal tract.

Ultrafiltration and equilibrium dialysis experiments showed that roughly 70% of the cyclamate in human plasma was bound to the proteins, but only 46% of the cyclamate circulating in rat plasma was protein bound.[337,401-403] Kojima and Ichibagase[404] found that the binding of cyclamate to bovine serum albumin was predominately reversible and the strength of its binding was weak.

Taylor et al.[393] were the first investigators to study the distribution of cyclamate by administering ^{35}S-cyclamate to rats and dogs. Radioactivity was found in most of the tissues of the body, with the highest levels initially in the kidneys and the lowest levels in the brain. In dogs given ^{14}C-cyclamate, relatively high levels of radioactivity were found in the kidney, liver, spleen, lungs, pancreas, and heart while the brain, testes, thyroid, eyes, and muscle had extremely low levels.[396] After a 2 hr equilibrium period, the levels of ^{14}C-cyclamate in the tissues of rats with ligated renal arteries were lower than the plasma concentrations in all organs except the liver.[403] The liver-to-plasma water ratio was 1.6 while other major tissues had ratios of 0.9 to 0.3. Almost all of the radioactivity in the brain was attributable to the residual blood in that organ. The apparent volume of distribution of cyclamate was estimated to be 0.57 ℓ/kg, which is similar to the body-water content.[403]

Cyclamate can also cross the placenta and enter the developing fetus in rat, monkey, and human.[393,405-408] In rhesus monkeys given ^{14}C-cyclamate during the last trimester of pregnancy, the maternal-to-fetal ratio of radioactivity in the blood was approximately 4:1, suggesting limited distribution to the fetus.[405] In early human pregnancy, cyclamate was also present in the fetal circulation at about one fourth of the maternal level.[406] The fetal tissues with the highest concentrations were the liver, spleen, and kidneys, but the total amount of cyclamate in the fetus at maximal levels represented less than 1% of the maternal intravenous dose.

Cyclamate is also found in the milk of rats, dogs, and pigs.[180,409-411] Within a few hours after intravenous administration, the concentrations in the milk of rats and dogs generally exceeded those in blood.[410-411] The higher lacteal levels appeared to result from the rapid clearance of the cyclamate from the blood and its retention in milk.

Cyclamate is readily excreted in the urine, apparently by both glomerular filtration and tubular secretion.[83,412] The active secretion of cyclamate into the urine of rats was saturable, but was not inhibited by *p*-aminohippuric acid, the prototype for the acid secretory system.[412] Biliary excretion is not an important route of elimination for cyclamate, as rats and dogs generally secreted less than 1% of a cyclamate dose in the bile.[391,393-394] Hence, the cyclamate eliminated in the feces after oral administration primarily represents unabsorbed compound.

Conversion of Cyclamate to Cyclohexylamine

Early studies indicated that cyclamate was not metabolized to an appreciable extent.[2,393,396]

However, in 1966 Kojima and Ichibagase[8] first reported the presence of cyclohexylamine in the urine of man and dog after ingestion of cyclamate. Subsequently, Oser et al.[33-34,413] also provided evidence for the conversion of cyclamate to cyclohexylamine in rats given a sodium cyclamate-sodium saccharin (10:1) mixture (2.5 g/kg/day) for 27 weeks. Since the original report, cyclohexylamine has been found in the urine of rats,[150,391,394,400,414-422] rabbits,[391,419-420] guinea pigs,[391,423-424] mice,[425] dogs,[62,426] pigs,[427] monkeys,[49,397,427] and humans[8,81-82,148,150,391,399,400,417,428-435] ingesting cyclamate, and many studies have been performed to elucidate the site and extent of the conversion of cyclamate to cyclohexylamine.

Sonders et al.[400] and Renwick and Williams[436] provided early evidence that cyclohexylamine was formed in the gastrointestinal tract. In the studies by Sonders et al.,[400] a rat, which had been maintained on a 5% sodium cyclamate diet, was given ^{14}C-cyclamate at various times either orally or intravenously. After three oral doses 50 to 86% of the carbon-14 was recovered in the urine, and 39 to 62% of the urinary radioactivity was associated with cyclohexylamine. However, following intravenous administration 87 to 100% of the ^{14}C-dose was recovered in the urine, and cyclohexylamine accounted for only 0 to 2% of the radioactivity. Several other studies confirmed that parenterally administered cyclamate was not metabolized in rats, guinea pigs, or pigs, but cyclohexylamine was formed from orally administered cyclamate.[391,416,417,424]

The temporal pattern of the urinary excretion of cyclamate and cyclohexylamine by humans ingesting cyclamate also suggested that the metabolite was being formed in the gastrointestinal tract. Sonders et al.[150,388,400] reported that the urinary cyclamate levels remained elevated throughout an entire 2 week period (21 to 41% of the administered daily dose), while the cyclohexylamine levels climbed rapidly from 1.4% of the cyclamate dose on the 1st day to a peak of 41% on the 4th day of treatment. If increasing amounts of cyclohexylamine were formed from the absorbed cyclamate in the liver, kidneys, or other organs, the urinary cyclamate should have fallen during the first 4 days of the study as the cyclohexylamine levels rose. Such, however, was not the case. Furthermore, in another subject the urinary cyclamate levels fell sharply after termination of treatment with cyclamate, but the cyclohexylamine levels in the urine remained fairly constant for the next 24 hr and only then started to decline. Similarly, Collings[417] reported that, after stopping the ingestion of cyclamate, it took at least 1 day before the urinary cyclohexylamine levels started to decrease and another 2 days before complete elimination occurred. This pattern of excretion also indicated that cyclohexylamine was slowly formed from the unabsorbed cyclamate in the gastrointestinal tract, rather than from the absorbed cyclamate present in the rest of the body.

Identification of the gastrointestinal tract as the site of conversion suggested the involvement of the gut microflora in the metabolism of cyclamate. Thus, it was hypothesized that a reduction in the microflora after treatment with antibiotics should result in a decrease in the formation of cyclohexylamine. Sonders et al.[400] found that the administration of neomycin or erythromycin to rats that were able to convert cyclamate to cyclohexylamine did, indeed, lead to a marked decrease in the urinary excretion of cyclohexylamine. Upon withdrawal of the antibiotics, cyclohexylamine excretion returned to the pretreatment levels within a few days. Other investigators subsequently confirmed that the administration of antibiotics to rats, guinea pigs, pigs, or man receiving cyclamate significantly decreased the formation and excretion of cyclohexylamine.[416,417,424]

In vitro studies further demonstrated that the intestinal microflora are responsible for the conversion of cyclamate to cyclohexylamine. Anaerobic incubation of cyclamate with intestinal contents or fecal homogenates from rats,[391,416,418,422,437,438] guinea pigs,[424,438,439] rabbits,[437-438] pigs,[417] dogs,[426] or man[437-438] led to the formation of cyclohexylamine. In contrast, tissue homogenates (e.g., liver, kidney, and spleen) from rats,[437-438] rabbits,[437,438] or guinea pigs[424] and isolated perfused rat livers[421] were unable to metabolize cyclamate, even though some of the tissues were taken from animals known to convert cyclamate to

cyclohexylamine in vivo. In one early study,[420] small quantities of cyclohexylamine, cyclohexanol, and cyclohexanone were detected in rat liver homogenates which had been incubated with cyclamate. However, since the rats had been pretreated with cyclamate for 1 week, it was not clear whether the metabolites were formed in vitro by the homogenates or were already present as a result of pretreatment with orally administered cyclamate.

Other investigators have attempted to identify the microorganisms responsible for converting cyclamate to cyclohexylamine, and it appears that a wide variety of bacteria are capable of metabolizing cyclamate. Drasar et al.[437-438] isolated clostridia from rat feces and demonstrated that the numbers of these organisms increased when the animals were kept on a cyclamate-containing diet for a prolonged period of time. Likewise, clostridia were identified as the converting organisms present in the large intestine of the dog.[426] Pseudomonas,[439,442] corynebacteria,[439] clostridria,[440-441] propionibacteria,[440-441] and campylobacter[440-441] have all been isolated from guinea pig feces, while the converting activity in rabbit fecal contents was associated with clostridria and enterobacteria in one study[437-438] and with clostridria, *Streptococcus faecalis,* Bacillus, and *Escherichia coli* in another study.[443-444] The active organisms in monkey feces appeared to be clostridria, lactobacilli, streptococci, and Bacteroidaceae.[445-446] Two groups of investigators[146,437-438] have identified enterococci as the bacteria capable of metabolizing cyclamate in human feces, but it is possible that other converting organisms may also be present in the human gastrointestinal tract.

Several factors have been shown to affect the ability of bacterial preparations to convert cyclamate to cyclohexylamine. The activity of a preparation from rat feces was enhanced by preincubation with cyclamate, but inhibited by cyclohexylamine. In contrast, preparations from rabbit or human feces were inhibited by cyclamate, but not by cyclohexylamine.[438] In addition to possible substrate and end-product inhibition, the activity of rat fecal preparations was inhibited by the sulfur containing amino acid cysteine, but not by other sources of sulfur, such as methionine, cystine, or sulfate.[422,447] Furthermore, cysteine also inhibited the incorporation of ^{35}S from radiolabeled cyclamate into protein by bacteria from rat feces.[448] Although in vivo studies have apparently not been performed, these findings suggest that the conversion of cyclamate to cyclohexylamine may be dependent on the levels of cysteine in the intestine and hence be subject to modification by dietary changes.

The enzyme capable of converting cyclamate to cyclohexylamine has been partially purified from extracts of *Pseudomonas* isolated from guinea pig feces.[442-443] The reaction was shown to be hydrolytic rather than reductive, and the enzyme was classified as sulfamatase. The Km for cyclamate was 5×10^{-3} *M*, which agreed reasonably well with the Km of 1.7×10^{-3} reported for a crude enzyme preparation from rat feces.[296] The pH optimum for the reaction was around 6.5 to 6.7. EDTA caused a loss of activity, which could be partially restored by various metal ions, and some sulfhydryl reagents were also inhibitory. A study of the substrate specificities indicated that the enzyme preferentially hydrolyzed aliphatic sulfamates with three to eight carbons, with the maximal rate occurring with the C_8 homolog. Among the six carbon sulfamates, the straight chain compound was more readily hydrolyzed than the cyclohexyl compound while *N*-phenyl sulfamate and sulfamates of secondary amines were not hydrolyzed appreciably. In contrast, Renwick[422] found that a rat fecal preparation readily metabolized phenyl sulfamate in addition to aliphatic sulfamates.

Generally, similar trends were observed in rat metabolism studies with a variety of sulfamates.[426,449-452] Cyclic sulfamates with five to eight carbons were metabolized, with the cyclooctyl compound apparently showing the greatest conversion in vivo. Methyl-substituted cyclohexyl derivatives were also metabolized, but the introduction of a double bond into the ring appeared to reduce the metabolism. However, considering the low levels of conversion observed in these studies and the variability reported for the conversion of cyclamate to cyclohexylamine, the quantitative comparisons may be somewhat tenuous. Renwick[422] demonstrated that cyclamate metabolizing rats could also convert 3-methylpentylsulfamate

to 3-methylpentylamine, thus confirming that the cyclamate hydrolyzing bacteria can handle other substrates in vivo.

One of the unique features of cyclamate metabolism is the induction of the conversion by continuous exposure to cyclamate. A single dose of cyclamate will frequently not be metabolized, but if animals are maintained on cyclamate they will often, although not always, acquire the ability to convert cyclamate to cyclohexylamine. For example, Bickel et al.[416] gave five groups of rats cyclamate in the drinking water (0.5% or about 100 mg/day) for 6 to 15 months. A total of 24 out of 26 rats became converters and excreted from 1 to 70% of the dose as cyclohexylamine. The time required for the development of the converting ability varied from 1 to 7 months of treatment. Renwick[422] also gave rats cyclamate in the drinking water, but found only a very gradual development of the ability to convert cyclamate to cyclohexylamine. After about 1 year, 50 to 60% of the animals had become good converters ($\geq$0.5%), but the average conversion was only about 1%. These results were in marked contrast to an earlier study by the same investigator in which most rats became converters within 3 months and conversion ranged from 0.5 to 35%.[391]

Dalderup et al.[418] originally suggested that coprophagia might be involved in transferring the converting ability from one rat to another, and several other investigators subsequently demonstrated that the converting ability could be transferred by housing nonconverting rats with converters or by the forced feeding of fecal material.[416-417] For example, Collings[417] fed 100 rats a diet containing cyclamate for 6 months without any of the animals acquiring the ability to convert cyclamate to cyclohexylamine. When four imported converter rats were housed with the nonconverters, the rats acquired the ability to metabolize cyclamate within 3 days. Bickel et al.[416] also found that normal rats became converters in a few days after being given feces from converters. When the fecal organisms were killed by heat or when feces from nonconverting rats were used, the rats did not become converters. However, this technique is not always sufficient as Renwick[422] was unable to transfer the converting ability to nonconverter rats either by housing the animals with converters or by treating them with a suspension of feces from the converters. Converting ability has also been transferred in mice, guinea pigs, rabbits, and monkeys by the forced feeding of feces or bacteria isolated from the feces of converting animals.[423,427,440,453]

The continuous administration of cyclamate is necessary not only to induce its own metabolism, but also to maintain the conversion capacity at a high level. If cyclamate is withdrawn from the diet, the ability to metabolize cyclamate is diminished and gradually lost. In rats, removal of cyclamate from the diet resulted in a loss of the conversion capacity within 1 to 2 weeks, and the repeated administration of cyclamate was again required for the animals to regain the ability to form large amounts of cyclohexylamine.[391,416] Similarly, human converters quickly lost the ability to metabolize cyclamate when the ingestion of cyclamate was stopped.[388,391,400] For example, after receiving cyclamate daily for 4 days, a human converter was able to metabolize 30% of the daily dose to cyclohexylamine. However, after abstaining from cyclamate for 5 days, only 1.5% of a single oral dose of cyclamate was converted to cyclohexylamine.[388,400] The need for continuous ingestion of cyclamate to sustain high levels of conversion is particularly significant since the dietary intake of cyclamate would often be sporadic and hence would tend to limit the conversion ability of the subject.

Another prominant feature of cyclamate metabolism in both animals and man is the great variability in the extent of conversion. Even with repeated administration of cyclamate, all animals do not acquire the ability to convert cyclamate to cyclohexylamine, and among the converters the extent of conversion varies greatly, both from animal to animal and from day to day. In groups of rats receiving daily doses of cyclamate for extended periods of time (6 to 15 months), the incidence of converters has ranged from 0 to 90%.[33,391,394,400,413,416-417,421-422] At least two investigators have reported that very few (0 to

4%) of the rats in their colonies acquired the ability to form cyclohexylamine even when given cyclamate in the diet for 6 to 10 months.[400,417] However, in other laboratories,[33,391,394,413,416,421-422] around 30 to over 90% of the animals became converters. The ability to convert cyclamate to cyclohexylamine appeared to be greater in the rats given the higher doses and also increased as the duration of the feeding period was extended.[413]

Among the converting rats, the percentage of the cyclamate dose metabolized to cyclohexylamine was quite variable. Oser et al.[413] found cyclohexylamine in urine samples from 20 of 60 rats receiving a cyclamate-saccharin mixture for 27 weeks; 10% of the rats excreted less than 0.1% of the dose as cyclohexylamine, 15% excreted from 0.1 to 1%, and 8% excreted from 1 to 10%. When the same animals were tested a few weeks later, the low converting animals generally remained low and the high converters (>1%) remained high. Wallace et al.[394] studied the metabolism of ^{14}C-cyclamate in rats that had received sodium or calcium cyclamate in the diet for at least 1 year. Of the 63 rats, 52 (83%) excreted ^{14}C-cyclohexylamine in the urine; 22 (35%) excreted less than 0.1%, 18 (29%) excreted 0.1 to 1.0%, and 12 (19%) excreted 1 to 38%. However, tremendous variations were observed in the amount of cyclohexylamine excreted in the urine by the high converters at different times. For example, one animal converted 38% of the first ^{14}C-cyclamate does to cyclohexylamine, but 9 weeks later metabolized only 0.33%. In contrast, the urinary excretion of cyclohexylamine by another rat increased from 8% of the first dose to 28% of the second dose.

Sonders et al.[400] followed the urinary excretion of cyclohexylamine by a rat that was given 5% cyclamate in the diet for 50 days. Very little cyclohexylamine was excreted in the urine on the 1st day of the study (1.5 mg); and then cyclohexylamine excretion gradually rose to 10.6 mg on the 6th day. The highest level of cyclohexylamine in the urine, 19.1 mg, occurred on day 33, but 6 days later the urinary cyclohexylamine decreased to 1.7 mg, despite the continued administration of cyclamate. Similarly, this rat excreted 11.4 mg of cyclohexylamine on day 22, 4 days after excreting 1.5 mg. The urinary excretion of cyclohexylamine during this study averaged 6.7 mg, or only about one third of the maximal level of cyclohexylamine in the daily urine samples. Relatively high cyclohexylamine levels were often preceded or followed by very low levels, despite continuous treatment with cyclamate.

Great variability has also been observed in the conversion of cyclamate to cyclohexylamine in man. Several studies have attempted to define the incidence of converters among human subjects ingesting cyclamate, and a summary of these studies is presented in Table 22. There were, of course, many differences in the experimental designs of these studies, including differences in the size and geographical area of the population studied, the dosage regimen (single vs. multiple dosing), the time of sample collection, and the types and sensitivities of the analytical techniques. In all of the studies combined, only about 300 out of a total of over 1200 subjects studied were able to convert cyclamate to cyclohexylamine, corresponding to an average incidence of 25%. In the studies involving North American or Western European subjects that received at least three daily doses of cyclamate, approximately 20% of the population converted cyclamate to cyclohexylamine.[81,399,417,430,433-434] The incidence of converters was higher among the Japanese and usually exceeded 80%.[8,428,432] The ability of most Japanese to convert cyclamate to cyclohexylamine is of interest in relation to the identification of enterococci as the converting organisms in human feces. According to Hill et al.,[8] the feces of normal Japanese contained an average 10^8 enterococci per g whereas fewer of these bacteria (10^5 per g) were found in the feces of British and American subjects. The extent of conversion by a given individual also varies greatly from day to day. With repeated administration of cyclamate, the amount of cyclohexylamine excreted in the urine generally increases over a period of at least 3 to 5 days,[150,417,430] or even longer,[391,433,435] and then reaches a plateau that is still subject to large daily fluctuations. Sonders et al.[150]

Table 22
INCIDENCE OF CYCLAMATE CONVERSION IN MAN

Ref.	Number of subjects	Number of converters	% Converters
Asahina et al.[428]	6	4	67
	50	43	86
Blumberg and Heaton[429]	83	31	37
	64	5	8
Collings[417]	141	36	26
Davis et al.[430]	11	11	100
Glogner[431]	100	12	12
	16	4	25
Hengstmann et al.[454]	255	89	35
Kojima and Ichibagase[8,432]	6	6	100
Leahy et al.[433-434]	40	5	12
Litchfield and Swan[148]	69	10	14
Pawan[435]	104	9	9
	52	8	15
Renwick and Williams[391]	3	1	33
Sonders et al.[150,388,399-400]	150	19	13
	49	4	8
Wills et al.[81]	24	10	42
Total	1223	307	25

reported that three of four individuals consuming 3 to 5 g of cyclamate a day for 4 days formed large amounts of cyclohexylamine, but exhibited substantial differences in the percent converted when monitored again several months later. Conversion decreased from 41 to 6% in one subject, and decreased from 38 to 18% in another, but increased from 6 to 31% in a third. The fourth subject, initially a low-level converter (0.4%), became a nonconverter in the subsequent analysis. Determination of the cyclohexylamine levels in the 24-hr urine samples from one of these subjects over several consecutive days further illustrated the large variations in the daily excretion levels. The urinary cyclohexylamine levels increased from 1.4% of the cyclamate dose on day 1 to 41.4% on day 4, then decreased to 12.9% on day 9 and subsequently increased again to 35.3%. Similar variations in the urinary excretion patterns were observed by Collings[417] and Davis et al.[430] Thus, although an individual may form and excrete large amounts of cyclohexylamine in the urine on a given day, this high level of conversion would probably not be maintained over an extended period of time. Therefore, an average value provides a better estimation of the conversion of cyclamate to cyclohexylamine that is likely during periods of dietary intake of cyclamate than the maximal urinary cyclohexylamine level found on a single day.

The average cyclohexylamine conversion values still exhibit great inter-subject variability. Among a group of 45 subjects who had been followed for several days, the average excretion values ranged from <0.01 to 62%. The 62% conversion probably represents the maximal possible formation of cyclohexylamine, since about 40% of the cyclamate is absorbed[83,391,399] and only the nonabsorbed portion would be available for metabolism by the intestinal flora. However, relatively few subjects form cyclohexylamine at anything close to this maximal rate. About $^1/_3$ of this group of 45 converters metabolized less than 1% of the dose to cyclamate and about $^1/_2$ converted less than 5%.

The average percent conversion among this group of 45 subjects was 12.6%. However, inclusion of a number of "low converters" would bias the average toward a lower level of conversion. Based only on the subjects that excreted $\geq 1\%$ of the dose as cyclohexylamine, the average conversion was 18.8%, and among the subjects excreting $\geq 5\%$, conversion averaged 24.6%. Thus, 20 to 25% would provide a good estimate of the average conversion

of cyclamate to cyclohexylamine among the high converters. It must again be emphasized that these high converters represent only a small segment of the population. Renwick[456] concluded that about 10% of an American or European population would metabolize more than 1% of the dose to cyclohexylamine.

The above analysis was based on the conversion of daily doses of cyclamate ranging from 250 mg to 5 g, and the data for all doses were combined. There is, however, an indication that the percent conversion may be dose-dependent. The highest percent conversion values tended to occur at the lower doses, and the average conversion values were generally lower with the higher doses. Collings[417] observed this trend in one of his studies with four converters who were given daily doses of 250, 500, or 1000 mg sodium cyclamate for 2-week periods. Based on the excretion during the last 6 days at each dose, the amount (milligrams) of cyclohexylamine increased, but the percentage conversion decreased. A similar inverse relationship was also noted by Litchfield and Swan[148] and by Davis.[430] This inverse relationship would be consistent with the in vitro inhibition of conversion in human fecal preparations by high cyclamate concentrations[438] and also with the hypothesis that conversion may be limited by the sulfur requirement of the microflora.[447-448] However, a definitive study is needed to confirm or negate this trend.

All of the previously discussed conversion data were based on the amount of cyclohexylamine excreted in the urine. In order for urinary cyclohexylamine levels to provide an accurate estimation of the metabolism of cyclamate to cyclohexylamine, the cyclohexylamine formed in the gastrointestinal tract must be well absorbed, not secreted in the bile, and eliminated by the kidneys. However, absorption must occur at the site of formation of cyclohexylamine, i.e., the cecum, colon, and rectum, and not in the small intestine where orally administered cyclohexylamine is probably absorbed. Drasar et al.[438] measured the appearance of radioactivity in the urine of rats after injection of ^{14}C-cyclohexylamine into the colon or cecum and found that cyclohexylamine was readily absorbed. Absorption of cyclohexylamine was also demonstrated by Asahina[423] following the injection of cyclohexylamine into the cecum of guinea pigs. In man, the cyclohexylamine formed from cyclamate also appears to be well absorbed since the reported levels of cyclohexylamine in the feces ranged from <1 to 6% of the cyclamate dose and averaged about 2%.[150,400,417,430] There is no evidence to suggest that the body is ever exposed to the small amounts of cyclohexylamine excreted in the feces, since biliary secretion does not appear to be a significant route of cyclohexylamine elimination. The cyclohexylamine in the feces probably represents a small amount of unabsorbed cyclohexylamine or cyclohexylamine formed by the bacterial metabolism of cyclamate in the feces after voiding.

Cyclohexylamine was the principal metabolite of cyclamate in all species studied, including man, dog, guinea pig, monkey, rabbit, and rat. Other metabolites were present in very small amounts and probably resulted from the further metabolism of cyclohexylamine. Urinary metabolites other than cyclohexylamine, included cyclohexanol and cyclohexanone, which were found in man,[150,388,399-400,426,432] rat,[419-420] monkey,[49,397] and rabbit,[419-420] while cyclohexanol, but not cyclohexanone, was found in guinea pigs.[424] The aminocyclohexanols or their conjugates, known to be metabolites of exogenously administered cyclohexylamine (Table 2), are probably also excreted in the urine of rats given cyclamate.[391,400] Dicyclohexylamine was reported to be a metabolite of cyclamate in rats and rabbits[421,458] while *N*-hydroxycyclohexylamine was reportedly found in the urine of monkeys and man.[397,426] However, the presence of these two compounds was not adequately demonstrated nor were the findings confirmed by most other investigators.

D. Cyclohexylamine

In comparison with cyclamate, cyclohexylamine is better absorbed from the gastrointestinal tract, has a shorter half-life in plasma, is less extensively bound to the plasma proteins, and is more widely distributed throughout the tissues of the body.

Several studies have demonstrated that orally administered cyclohexylamine is rapidly and well absorbed in both animals and man.[145-146,457,459-460] After an oral dose of the radiolabeled compound, about 90% or more of the ^{14}C-dose was eliminated in the urine of rat, dog, guinea pig, rabbit, and man, thus indicating nearly complete absorption from the gastrointestinal tract. In rats and dogs, the peak cyclohexylamine levels in blood or plasma were achieved within the 1st hr, and the half-life was about 1 to 2 hr in rat and 3 hr in dogs.[144,460] In man, the peak blood or plasma levels occurred between 1 and 2 hr and the half-life ranged from 3 to 5 hr.[145-146,460]

It, however, must be realized that this pattern of high peak and rapidly declining cyclohexylamine levels seen after an oral dose of the compound would not be typical of that found after ingestion of cyclamate. In the latter case, the cyclohexylamine would be slowly formed from the nonabsorbed cyclamate, and hence, the circulating cyclohexylamine levels would probably be quite low and sustained. Unfortunately, there is little information available about the circulating cyclohexylamine levels found in animals or man after the ingestion of cyclamate. Collings[417] reported cyclohexylamine blood levels of 0.2 mcg/mℓ in human converters ingesting cyclamate and excreting approximately 4 mg cyclohexylamine per kilogram per day. This concentration is in reasonably good agreement with the levels predicted from the pharmacokinetics of cyclohexylamine with the assumption of zero-order absorption over a 24-hr period (see Section III.B.2.a.). The circulating cyclohexylamine levels found in rats ingesting diets containing cyclohexylamine would probably show yet another pattern. Since rats tend to eat at a few discrete times during the dark period, their cyclohexylamine blood levels would probably rise quickly after eating, then decline rapidly, and remain low until the next feeding period. Hence, experimentally determined blood levels would be dependent upon the time of sampling relative to the animals' ingestion of food. These differences in the kinetics of the circulating cyclohexylamine levels and the paucity of experimental data make it difficult to correlate the cyclohexylamine levels in animal toxicity studies with those occurring in humans ingesting cyclamate.

In rats, cyclohexylamine readily penetrates into the body tissues, with the highest concentrations occurring in the lungs, spleen, liver, adrenals, heart, gastrointestinal tract, and kidneys.[461] Consistent with the basic nature of the compound, the levels in most tissues were higher than those in plasma. The apparent volume of distribution in rats was calculated to be 2.7 ℓ/kg,[460,461] which agreed well with the 2.1 to 2.9 ℓ/kg values reported for man.[146] In rats only 8% of the cyclohexylamine was bound to the plasma proteins,[460] while the binding to human serum albumin averaged 33% at 5 mcg/mℓ.[146] Cyclohexylamine also freely diffuses across the placenta and enters the fetus. After administration of ^{14}C-cyclohexylamine to pregnant rhesus monkeys, the levels of radioactivity in the maternal and fetal blood were virtually identical, in contrast to the 4:1 ratio seen with cyclamate.[405]

Cyclohexylamine is readily excreted in the urine, and the renal elimination of the unchanged drug probably accounts for at least 80 to 90% of the dose in most species. In man the renal clearance values exceeded the creatinine clearance, indicating that cyclohexylamine was probably removed by tubular secretion as well as glomerular filtration.[146] The renal clearance of cyclohexylamine decreased as the dose increased (2.5 to 10 mg/kg), suggesting that the secretion process might be easily saturated.

Renwick and Williams[457] found that less than 10% of a ^{14}C-cyclohexylamine dose was metabolized in female rats and guinea pigs, while about 30% was metabolized in rabbits. However, only 1 to 2% of the orally administered ^{14}C-cyclohexylamine was metabolized in man.[457,460] The principal metabolic pathway in rats involved ring hydroxylation, leading to isomers of 3- or 4-aminocyclohexanol.[457] Only the deaminated products, cyclohexanol, and *trans*-cyclohexane-1,2-diol, were found in man, while both deamination and ring hydroxylation occurred in guinea pigs and rabbits.[457] The deaminated products, cyclohexanone and cyclohexanol, have been identified in dogs,[459] but no definitive information is available

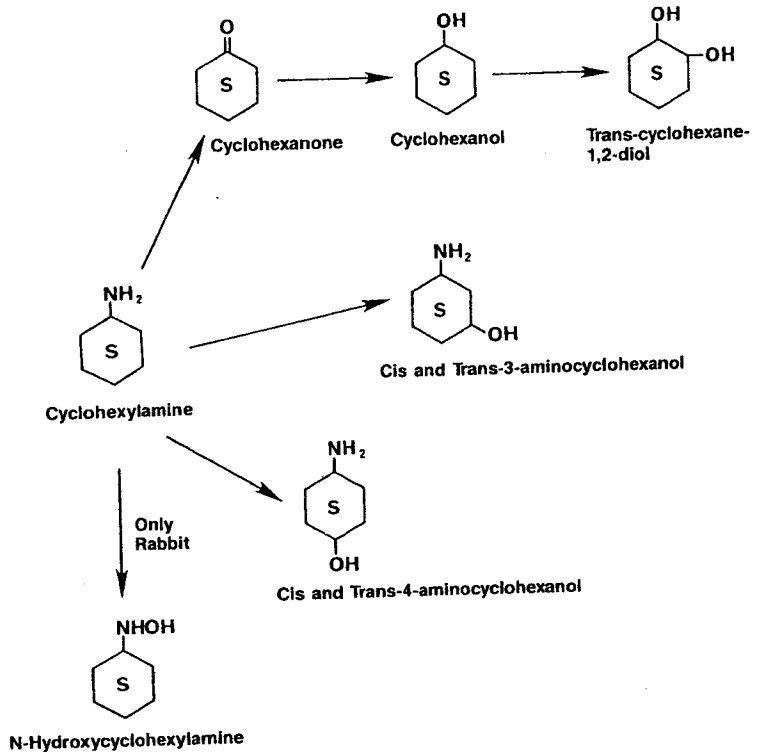

FIGURE 3. Metabolic fate of cyclohexylamine.

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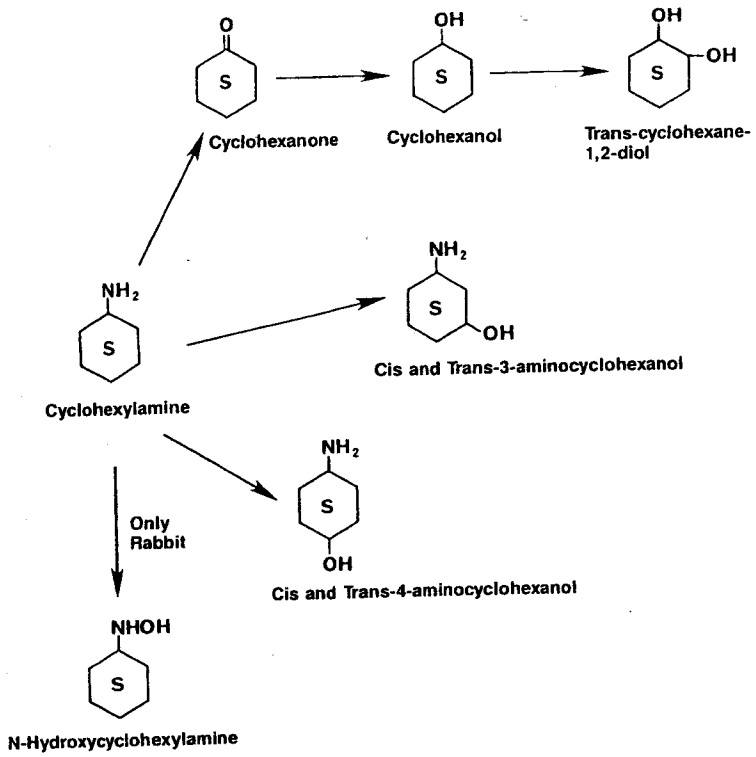

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Several studies have demonstrated that orally administered cyclohexylami and well absorbed in both animals and man.[145-146,457,459-460] After an oral dose labeled compound, about 90% or more of the ^{14}C-dose was eliminated in the dog, guinea pig, rabbit, and man, thus indicating nearly complete absorpt gastrointestinal tract. In rats and dogs, the peak cyclohexylamine levels in blo were achieved within the 1st hr, and the half-life was about 1 to 2 hr in ra dogs.[144,460] In man, the peak blood or plasma levels occurred between 1 and half-life ranged from 3 to 5 hr.[145-146,460]

It, however, must be realized that this pattern of high peak and rapidly dec hexylamine levels seen after an oral dose of the compound would not be typical after ingestion of cyclamate. In the latter case, the cyclohexylamine would be sl from the nonabsorbed cyclamate, and hence, the circulating cyclohexylamine probably be quite low and sustained. Unfortunately, there is little information av the circulating cyclohexylamine levels found in animals or man after the ing clamate. Collings[417] reported cyclohexylamine blood levels of 0.2 mcg/mℓ in verters ingesting cyclamate and excreting approximately 4 mg cyclohexylamine per day. This concentration is in reasonably good agreement with the levels pr the pharmacokinetics of cyclohexylamine with the assumption of zero-order abs a 24-hr period (see Section III.B.2.a.). The circulating cyclohexylamine levels ingesting diets containing cyclohexylamine would probably show yet another p rats tend to eat at a few discrete times during the dark period, their cyclohexyl levels would probably rise quickly after eating, then decline rapidly, and rem the next feeding period. Hence, experimentally determined blood levels would b upon the time of sampling relative to the animals' ingestion of food. These d the kinetics of the circulating cyclohexylamine levels and the paucity of exper make it difficult to correlate the cyclohexylamine levels in animal toxicity studi occurring in humans ingesting cyclamate.

In rats, cyclohexylamine readily penetrates into the body tissues, with the centrations occurring in the lungs, spleen, liver, adrenals, heart, gastrointestin kidneys.[461] Consistent with the basic nature of the compound, the levels in most higher than those in plasma. The apparent volume of distribution in rats was be 2.7 ℓ/kg,[460,461] which agreed well with the 2.1 to 2.9 ℓ/kg values reported In rats only 8% of the cyclohexylamine was bound to the plasma proteins,[4] binding to human serum albumin averaged 33% at 5 mcg/mℓ.[146] Cyclohexylamin diffuses across the placenta and enters the fetus. After administration of ^{14}C-cycl to pregnant rhesus monkeys, the levels of radioactivity in the maternal and fetal virtually identical, in contrast to the 4:1 ratio seen with cyclamate.[405]

Cyclohexylamine is readily excreted in the urine, and the renal eliminatio changed drug probably accounts for at least 80 to 90% of the dose in most spe the renal clearance values exceeded the creatinine clearance, indicating that cycl was probably removed by tubular secretion as well as glomerular filtration.[1 clearance of cyclohexylamine decreased as the dose increased (2.5 to 10 mg/kg) that the secretion process might be easily saturated.

Renwick and Williams[457] found that less than 10% of a ^{14}C-cyclohexylamir metabolized in female rats and guinea pigs, while about 30% was metabolize However, only 1 to 2% of the orally administered ^{14}C-cyclohexylamine was me man.[457,460] The principal metabolic pathway in rats involved ring hydroxylation isomers of 3- or 4-aminocyclohexanol.[457] Only the deaminated products, cycloh *trans*-cyclohexane-1,2-diol, were found in man, while both deamination and r ylation occurred in guinea pigs and rabbits.[457] The deaminated products, cyclohe cyclohexanol, have been identified in dogs,[459] but no definitive information

since cyclohexylamine is known to chemically form salts with fatty acids.[468] Furthermore, the existence of this pathway in vivo has not been documented.

IX. SUMMARY

Cyclamate has been extensively studied in both animals and man. Its acute oral toxicity is of a very low order (10 to 20 g/kg), and even when doses in the gram per kilogram range are administered chronically to laboratory animals, cyclamate induces few pathophysiological changes. The only effect seen in all animal species, including man, is the development of soft stools, which result from the osmotic activity of the unabsorbed cyclamate and can progress to diarrhea if the dose is raised high enough. With chronic ingestion of food containing 5% sodium or calcium cyclamate (approximately 2.5 g/kg/day), rats occasionally exhibit increased incidences of renal disorders, especially nephrocalcinosis, in addition to testicular atrophy. The latter does not appear to be a direct toxic effect of cyclamate, but more often develops in aging rats secondarily to body weight reductions and nutritional deficiencies. In mice, the chronic administration of 7% sodium cyclamate in the diet (about 10 g/kg/day) resulted in the development of a mild anemia. In hamsters, very high doses of cyclamate were associated with myocardial or vascular calcification, but these effects appear to be species specific. Far more impressive than the few adverse effects that are seen is the relative absence of any consistent severe toxic manifestations from the chronic administration of high doses of cyclamate to laboratory animals.

Reproduction studies demonstrated that cyclamate is not teratogenic in mammals, and the occasional reductions in the survival or growth of rat pups apparently result from decreased lactation associated with reductions in the body weight of the cyclamate-treated dams.

Cyclohexylamine, the major metabolite of cyclamate, has also undergone extensive toxicity testing. It is considerably more toxic than cyclamate, with acute LD_{50} values 20 to 50 times lower than those for cyclamate. The organ that is most sensitive to the toxicological effects of cyclohexylamine is the testes. The administration of cyclohexylamine to rats causes testicular atrophy, characterized by a reduction in the absolute weight of the testes and a marked impairment in spermatogenesis in the affected seminiferous tubules. A 3-month feeding study conducted by Brune et al.[162] demonstrated that the no-adverse effect dose in rats is at least 100 mg/kg/day. Similar testicular changes were not seen in mice given cyclohexylamine in doses up to 300 mg/kg/day.

Cyclohexylamine is an indirectly acting, sympathomimetic agent similar to tyramine, but many times less potent. Although cyclohexylamine possesses the inherent ability to increase blood pressure, hypertension does not appear to develop in animals given cyclohexylamine chronically or in animals and humans ingesting high doses of cyclamate.

Reproduction studies in mice and rats indicated that the administration of high doses of cyclohexylamine may be associated with slight decreases in the number of pups born alive, placental and fetal weights, pup survival, and pup growth, but these effects were generally accompanied by, and were probably secondary to, reductions in the body weight of the dams. Cyclohexylamine was not teratogenic in any of the studies with mice, rats, or monkeys.

The most serious question about the safety of cyclamate arose in 1969 when the results of a study by Oser et al.[33] implicated cyclamate as a bladder carcinogen in rats. Subsequently, bioassays were initiated in many of the leading carcinogenicity testing laboratories throughout the world. These studies in mice, rats, and hamsters failed to confirm the earlier findings and instead demonstrated that neither cyclamate nor cyclohexylamine is carcinogenic. Long-term studies in dogs and monkeys support that conclusion, and epidemiology studies have not demonstrated an increased risk of human bladder cancer associated with the use of artificial sweeteners. In spite of the large number of negative carcinogenicity studies, some questions have still been raised about possible increases in the incidences of bladder tumors

or hepatic, pulmonary, and lymphatic tumors in mice. However, after careful evalof all the data, most scientific groups and regulatory agencies throughout the world ncluded that cyclamate and cyclohexylamine are not carcinogenic.

nutagenic potential of cyclamate and cyclohexylamine has been evaluated in a variety systems. Both compounds are inactive in the "Ames" test with Salmonella. Positive gative results have been obtained in cytogenetic studies with somatic cells, but the findings were restricted to an increased incidence of gaps and breaks, which may flection of nonspecific cytotoxicity. There was no evidence of treatment-related es in the incidence of exchange figures and translocations, which are generally con- to be better indicators of true genetic damage. The results of the cytogenetic studies cells and dominant lethal assays were largely negative. When the entire battery of evaluated, the evidence suggests that neither cyclamate nor cyclohexylamine rep- a mutagenic hazard.

e cyclohexylamine is more toxic than cyclamate, the extent of metabolism becomes al issue in establishing the safety of the artificial sweetener. Cyclamate is not med by mammalian tissues, but rather the cyclohexylamine is formed from the nond cyclamate by the bacteria in the gastrointestinal tract. However, the conversion of ate to cyclohexylamine is extremely variable, both from individual to individual and lay to day. Only about one fourth of a human population possesses the ability to t cyclamate to cyclohexylamine, and the extent of conversion ranges from <0.1% to mum of about 60%. Conversion averaged about 20% in a group of subjects converting t 1% of a cyclamate dose to cyclohexylamine, but these good converters represent small segment of the population. Hence, most of the population would be exposed low amounts of cyclohexylamine, and considering both the no-adverse effect dose average conversion levels, even the good converters would have an adequate margin ty if cyclamate were used as an artificial sweetener. This is consistent with the extensive l safety studies performed with cyclamate in healthy adults, diabetics, and patients epatic, renal, or gastrointestinal disorders, as well as the history of safe use by the l public during the 2 decades when cyclamate was used extensively as an artificial ner and food additive in the U.S. and during its continued use in various countries ing Switzerland and Australia.

ACKNOWLEDGMENT

authors wish to thank Ms. Marcia Grissom for her excellent assistance in preparing anuscript.

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The mutagenic potential of cyclamate and cyclohexylamine has been evaluated in a variety of test systems. Both compounds are inactive in the "Ames" test with Salmonella. Positive and negative results have been obtained in cytogenetic studies with somatic cells, but the positive findings were restricted to an increased incidence of gaps and breaks, which may be a reflection of nonspecific cytotoxicity. There was no evidence of treatment-related increases in the incidence of exchange figures and translocations, which are generally considered to be better indicators of true genetic damage. The results of the cytogenetic studies in germ cells and dominant lethal assays were largely negative. When the entire battery of tests is evaluated, the evidence suggests that neither cyclamate nor cyclohexylamine represents a mutagenic hazard.

Since cyclohexylamine is more toxic than cyclamate, the extent of metabolism becomes a critical issue in establishing the safety of the artificial sweetener. Cyclamate is not metabolized by mammalian tissues, but rather the cyclohexylamine is formed from the nonabsorbed cyclamate by the bacteria in the gastrointestinal tract. However, the conversion of cyclamate to cyclohexylamine is extremely variable, both from individual to individual and from day to day. Only about one fourth of a human population possesses the ability to convert cyclamate to cyclohexylamine, and the extent of conversion ranges from <0.1% to a maximum of about 60%. Conversion averaged about 20% in a group of subjects converting at least 1% of a cyclamate dose to cyclohexylamine, but these good converters represent only a small segment of the population. Hence, most of the population would be exposed to very low amounts of cyclohexylamine, and considering both the no-adverse effect dose and the average conversion levels, even the good converters would have an adequate margin of safety if cyclamate were used as an artificial sweetener. This is consistent with the extensive clinical safety studies performed with cyclamate in healthy adults, diabetics, and patients with hepatic, renal, or gastrointestinal disorders, as well as the history of safe use by the general public during the 2 decades when cyclamate was used extensively as an artificial sweetener and food additive in the U.S. and during its continued use in various countries including Switzerland and Australia.

ACKNOWLEDGMENT

The authors wish to thank Ms. Marcia Grissom for her excellent assistance in preparing this manuscript.

Appendix 1
SOME CHEMICAL AND PHYSICAL PROPERTIES OF CYCLAMIC ACID, ITS SALTS, AND CYCLOHEXYLAMINE

Parameter	Cyclamic acid	Sodium cyclamate	Calcium cyclamate	Cyclohexylamine
Appearance	White crystalline powder, odorless	White crystalline powder, odorless	White crystalline powder, odorless	Clear colorless liquid, amine odor
Empirical formula	$C_6H_{13}NO_3S$	$C_6H_{12}NO_3S \cdot Na$	$C_{12}H_{24}N_2O_6S_2 \cdot Ca$	$C_6H_{11}NH_2$
Molecular weight	179.24	201.22	396.54 (432.58 as dihydrate)	99.17
Solubility (at approx. 25°C)				
Water	1 g/7.5 mℓ	1 g/5 mℓ	1 g/4 mℓ	Miscible
Alcohol	1 g/4 mℓ	1 g/250 mℓ	1 g/60 mℓ	Miscible
Propylene glycol	1 g/4 mℓ	1 g/25 mℓ	1 g/1.5 mℓ	
Chloroform	1 g/250 mℓ	Insoluble	Insoluble	Miscible
Melting or boiling point	170—180°C (MP)			134.5°C (BP)
pH	10% aq. soln. 0.8—1.6	10% aq. soln. 5.5—7.5	10% aq. soln. 5.5—7.5	0.01% aq. soln. 10.5

From Beck, K. M., *Food Technol.*, 11, 56, 1957; Beck, K. M., *CRC Handbook of Food Additives*, Vol. II, 2nd ed., Furia, T. E., Ed., CRC Press, Boca Raton, Fla., 1980, 125; International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Some Non-Nutritive Sweetening Agents*, Vol. 22, IARC, Lyon, 1980, 33; Theivagt, J. G., *Encycl. Ind. Chem. Anal.*, 11, 209, 1971; and Theivagt, J. G. et. al., *Encycl. Ind. Chem. Anal.*, 11, 220, 1971. With permission.

of tumors. However, the latter issue is perhaps less clear-cut in the lymphoreticular sys Some[24] have argued in favor of combining lymphosarcomas, reticular cell sarcomas certain leukemias while others[23] have argued against such a procedure. No dose-resp relationship is seen in the female mice from the Branton study if both the lymphosarc and reticular cell sarcomas are considered.

All these factors would affect the statistical significance calculated for the various parisons. However, the real issue should probably not be centered on these arguments. if a few of the comparisons approach or reach a level usually accepted for statistica nificance, doubts must remain[225] about the biological significance of a tumorigenic resp that occurs only in the females of one strain and the males of another, that is not repli in three generations of the same study, and that does not consistently demonstrate responsiveness. Moreover, the three groups of investigators had each independently cluded that cyclamate or cyclohexylamine did not exhibit a carcinogenic effect in studies, and other studies in mice and rats also gave no indication of such an effect.

F. Lung and Liver Tumors

The issue of lung and liver tumors in cyclamate-treated mice is based exclusively c study by Rudali et al. The NCI Committee that reviewed the cyclamate data conclude this study suffered from many deficiencies in the experimental design and the way in v it was conducted.[15] Most notably, the randomization techniques were not describec group sizes were small, histological examinations of the tumors were not performed the statistical analyses were apparently conducted on the total tumor incidence rather on a specific type of tumor. These weaknesses were confirmed by other governm agencies that evaluated the study,[24,225] and hence little weight has been given to Ru results.

The lung and liver tumors were each increased in only one of the strains of mice us this study. The liver tumors occurred in the F_1 males from a cross between C_3H and mice. That strain showed a substantial incidence of spontaneous liver tumors, thus m it more difficult to interpret the increase seen in the cyclamate-treated mice, espe without complete survival data. The lung tumors were found in female XVII/G mice, strain is apparently derived from the A strain, which is prone to the development of pulm tumors.[24] In the cyclamate study, the spontaneous incidence of lung tumors was re to be 19%, but in two later studies from the same laboratory it had increased to arou to 70%,[226-227] thus casting some doubt on the significance of the findings in the cycl treated mice. In contrast to Rudali's work, lung and liver tumors were not found in the other studies with cyclamate or cyclohexylamine in mice, all of which used more and higher doses, albeit in different strains.[45,59,60,127] Similarly, the incidence of pul and hepatic tumors was not increased in any of the studies with cyclamate or cyclohex in rats.[33-34,68-69,71-72,124-126] Thus, the many negative bioassays would appear to outwe questionable data from Rudali's study.

G. Cocarcinogenicity and Promotion Studies

The effects of cyclamate on the activity of several known carcinogens, includi zopyrene, 2-acetylaminofluorene, butylbutanolnitrosamine, and *N*-methylnitrosoure also been evaluated. In one of the first studies, Roe et al.[67] administered a single do of benzo[a]pyrene (50 mcg) to female Swiss mice 7 days before placing them on diets. Benzopyrene primarily induced papillomas and carcinomas of the forestoma the incidence and degree of malignancy of the tumors were not increased by sodium cy (5% in the diet). Ershoff and Bajwa[228] gave groups of 12 female Sprague-Dawley rats food containing 2-acetylaminofluorene (AAF; 300 mg/kg diet) with or without cyclamate (5% in the diet). After 40 weeks of treatment, mammary and ear duct

Table 13
PROMOTION OF MNU-INDUCED BLADDER TUMORS IN RATS GIVEN CYCLAMATE OR SACCHARIN

	Hicks et al.[209]		Green et al.[237]		
	Bladder tumors		Bladder tumors		Urinary tract tumors
Treatment	%	Onset[c] (Weeks)	%	Onset[d] (Weeks)	%[e]
None	0	—	0	—	1
Water[a]			2	50	2
MNU[a] (1.5 or 2.0 mg)	0	—	41	69(16—106)	57
MNU[a] + Cyclamate[b]					
2% or 1 g/kg	58	9			
4% or 2 g/kg	44	8	42	84(37—107)	70
MNU[a] + Saccharin[b]					
4% or 2 g/kg	47	8	38	77(14—107)	70
4 g/kg	57	8			
MNU[a] + $CaCO_3$[b] (3%)			39	82(26—107)	65

[a] Intravesical.
[b] Ingestion in food or water.
[c] Onset of first tumor.
[d] Mean and (range) of onset times.
[e] Including kidney, ureter, and bladder tumors.

were found in 92% of the rats given AAF alone, but only 2 of the 12 rats (17%) given AAF plus cyclamate had developed these tumors. The size and severity of the hepatomas induced by AAF also appeared to be reduced by cyclamate. In contrast, Rudali et al.[229] reported that cyclamate increased the frequency and decreased the latency period of the hepatomas caused by AAF (1 g/kg) in mice. However, cyclamate did not affect the incidence of irradiation-induced leukemia or mammary tumors induced by a contraceptive agent in mice.

Several of the cocarcinogenicity studies have directed attention toward the urinary bladder. Butylbutanolnitrosamine (10 mg/kg/day), administered to male Sprague-Dawley rats in the drinking water, induced squamous cell carcinomas in the urinary bladders of all animals, but coadministration of sodium cyclamate (2500 mg/kg/day) did not affect the time course of the carcinogenic response to the nitrosamine.[206] Aeschbacher et al.[230] gave male Swiss mice sodium nitrite and methylurea orally, thus leading to the formation of *N*-methylnitrosourea (MNU) in the stomach. Hyperplastic and neoplastic changes were not seen in the bladder of these mice after 3 months of treatment, but the MNU had caused focal epithelial proliferation in the lung, which was not enhanced by cyclamate administration.

The most controversial studies in this area involved the promotion of bladder tumors initiated by the intravesical instillation of MNU. In this model, which was developed by Hicks and her co-workers[208-209,231-234] in the 1970s, 1.5 to 2.0 mg of MNU was instilled directly into the urinary bladder of female Wistar rats, which were subsequently given sodium cyclamate (1 or 2 g/kg/day) or saccharin (2 or 4 g/kg/day) in the food or water. In her initial study, this dose of MNU alone did not cause any bladder tumors, but the incidence of tumors in the rats given MNU and cyclamate or saccharin was about 50% (Table 13). The first tumors were observed after only 8 to 9 weeks of treatment.

Green, Mohr, and their colleagues[235-238] attempted to replicate Hicks' work using a similar dose of MNU and a similar experimental design. Female Wistar rats were again given a single intravesical injection of 2.0 mg MNU and then were placed on the control diet or diet supplemented with sodium cyclamate (2%), saccharin (2%), or calcium carbonate (3%). The dietary concentrations of the sweeteners were increased to 4% after 10 weeks of treat-

ment. In this experiment, all the MNU-treated groups developed tumors in the urinary tract (bladder, ureters, and/or renal pelvis). The incidence of the urinary tract tumors in the groups receiving MNU with the test diets was slightly, but not significantly, greater than that in the rats given MNU alone (Table 13). Since the results with calcium carbonate, cyclamate and saccharin were similar, any effect that might exist must be nonspecific and could not be attributed to the sweeteners. The incidence of only the bladder tumors averaged about 40% in all the MNU groups, but the mean onset time was actually slightly longer in the rats given cyclamate or calcium carbonate than just MNU alone. Because the high incidence of tumors in the rats receiving MNU made it more difficult to demonstrate a promotional effect, special attention was directed toward the morphology of the lesions and their latency periods. However, even when the preneoplastic and neoplastic lesions were carefully graded histologically and their latencies analyzed, no effects attributable to cyclamate could be detected.[238] A further study of the development of bladder calculi in the rats given MNU and the artificial sweeteners failed to establish any correlation between the occurrence of stones and neoplasms.

Two major discrepancies are evident in the results of these two studies — whether 1.5 to 2.0 mg of MNU is a subcarcinogenic or carcinogenic dose and whether cyclamate enhances the carcinogenic activity of MNU or not. Mohr found about a 40% incidence of bladder tumors in rats given a 2.0 mg dose of MNU alone while Hicks found no tumors at all. The results of other studies are more consistent with Mohr's findings. Severs et al.[239] in Hicks' laboratory reported a 20% incidence of bladder tumors in rats given a single 1.5 mg dose of a different lot of MNU whereas Hooson et al.[240] found bladder neoplasms in 27 to 38% of their rats given a 1.5 mg dose of MNU as an intravesical instillation. Even a single 0.5 mg dose of MNU produced bladder tumors in about 16% of the rats after two years.[241] Hence, Hicks' claim that 1.5 to 2.0 mg of MNU was a subcarcinogenic dose is difficult to reconcile with any of the later studies. One possible explanation for this discrepancy is that the MNU used by Hicks in her first study might have been degraded since it is a relatively unstable compound.[23,24,239] Mohr and his colleagues[235-238] were similarly unable to confirm Hicks' demonstration of a promotional effect with cyclamate. It has been suggested[240] that Mohr's study does not represent a valid test since the dose of MNU was not subcarcinogenic, but the absence of any effect on the latency period or the severity of the lesions would certainly argue against cyclamate exerting a marked effect, such as that initially reported by Hicks.

In 1976 the NCI committee, which reviewed all the carcinogenicity studies with cyclamate, concluded that Hicks' method needed to be validated before it could be accepted as a technique for evaluating substances suspected of being bladder carcinogens.[15] To date, her results have not been confirmed in spite of Mohr's attempt to validate the model. Furthermore, this technique represents an artificial situation, which has questionable relevance to the human situation. Instillation of a toxic dose of a carcinogen directly into the bladder does not resemble human exposure, and the procedure, as well as the MNU, may well affect the integrity of the bladder epithelium. Hence, considering all factors, little reliance can be placed on this technique as a method for assessing the possible carcinogenic or promotional effects of cyclamate or any other substance.

Recently, at least two other models have been developed for investigating the initiation and promotion of urinary bladder carcinogenesis in rats. Cohen and his colleagues[242] have used *N*-[4-(nitro-2-furyl)-2-thiazoyl]formamide (FANFT) administered in the diet as the initiator, whereas Ito et al.[243] have employed *N*-butyl-*N*-(4-hydroxylbutyl)nitrosamine (BBN) administered in the drinking water. Cyclamate has apparently not been tested in the FANFT model, but Ito et al.[243] reported that sodium cyclamate (2.5% in the diet) did not significantly increase the papillary or nodular hyperplasia of the bladder induced by BBN. Partial cystectomy also failed to increase the incidence of hyperplasia in rats pretreated with BBN and given cyclamate (2.5%) in the diet for 10 weeks.[244]

Cyclamate has also been tested in several in vitro models for promotional activity. Ishii[245-246] found that high concentrations of cyclamate, like the tumor promoting phorbol esters, reversibly inhibited neurite outgrowth induced by nerve growth factor in embryonic chick ganglia. However, unlike the phorbol esters, cyclamate inhibited the binding of ^{125}I-labeled nerve growth factor to the ganglia cells. Lee[247-249] reported that high concentrations of cyclamate also inhibited the binding of ^{125}I-labeled mouse epidermal growth factor, multiplication stimulating activity, and insulin, but not human growth hormone or concanavalin A, to a variety of cell lines. The tumor promoter 12-*O*-tetra-decanoyl phorbol acetate was only active in the epidermal growth factor system. Shoyab and Todaro[250] used tritiated phorbol-12,13-dibutyrate (PDBu) to study the binding of the phorbol esters and found that the tumor-promoting esters inhibited the binding of PDBu to mink lung cells while the biologically inactive derivatives did not. Cyclamate failed to inhibit the binding of PDBu in their system[250] and also in human neuroblastoma cells.[251] Cyclamate promoted colony formation by viral-infected mouse $_3T_3$ cells, but was considerably less active than the tumor promoting phorbol esters.[252] Boyland[253-254] demonstrated that both cyclamate and cyclohexylamine reduced the interfacial tension between water and *n*-octanol, and attempted to correlate tumor promotion with surface activity. Other compounds, such as fatty acids, bile acids, and Tween-40, had much greater surface activity. Freedman et al.[225] investigated the induction and inhibition of aryl hydrocarbon hydroxylase in a human lymphocyte cell line since some work had suggested a relation between that enzyme and cancer susceptibility. Cyclamate did not induce aryl hydrocarbon hydroxylase in their cell culture system.

H. Other Studies

Two other studies should be mentioned for the sake of completeness, but both are of little value in assessing the potential carcinogenicity of a food additive. In 1970, Bryan and Ettuck[199] reported that the incidence of bladder carcinomas in mice with pellets containing sodium cyclamate implanted in the urinary bladder (61 to 78%) was greater than that in mice exposed to cholesterol pellets (12 to 13%). However, it is well established that the pellet plays a significant role in the development of the tumors and that the drug effects are actually being assessed against an increasing background incidence of tumors in the control animals.[214] This technique is no longer widely used for evaluating the carcinogenicity of test substances since it represents an unusual method of exposure and may give results different from those obtained with more conventional routes of administration.

Grasso et al.[256] observed the formation of local sarcomas in rats given repeated subcutaneous injections of calcium cyclamate. Sodium cyclamate failed to elicit any tumors, and hence the sarcomas were attributed to the high concentrations of the calcium ion. The surface activity of other chemicals was also found to be related to the induction of sarcomas at the injection site, and it was concluded that this technique was not valid for assessing the possible carcinogenicity of food additives.

I. Adequacy of the Carcinogenicity Studies

Whenever a compound generates as much interest and controversy as cyclamate has, the quality of the resultant studies tends to vary greatly. Also, whenever the safety evaluation of a compound covers a span of more than three decades, as cyclamate has, the older studies cannot be expected to meet the current standards. This problem is perhaps most acutely felt in the area of carcinogenicity testing, which has grown from its infancy in the 1950s into the highly specialized field known today. Moreover, the techniques used in carcinogenicity testing have frequently changed so rapidly that by the time a lifetime rodent bioassay is completed and reported, its experimental design may no longer satisfy all the current requirements. However, the answer to this dilemma is not found in arbitrarily disregarding all the older studies. The very nature of the scientific process dictates that new experiments build on and amplify the results of the older work. Hence, each study must be critically

examined, both for its individual merit and as it contributes to the overall knowledge about the compound. Furthermore, when so many studies, each involving numerous statistical comparisons, are performed, it is not surprising that anomolous or even spurious experimental results are occasionally obtained. These instances become evident when the entire picture is evaluated, and it is inappropriate to direct an inordinant amount of attention to isolated findings that cannot be replicated or reconciled with the rest of the data.

Cyclamate is certainly not immune from these problems. Probably no single carcinogenicity study would satisfy all of the current guidelines set forth by the regulatory agencies throughout the world.[24] Kroes'[60] three-generation study with cyclamate, cyclamate-saccharin, and cyclohexylamine would probably come the closest to meeting the standards of today. The three studies conducted at BIBRA (Brantom et al.[45] with cyclamate, Gaunt et al.[124] and Hardy et al.[127] with cyclohexylamine in rats and mice, respectively) are also notable, especially for the complete histopathological examinations. Although Schmähl's[68-69] two large studies in rats, one of which involved *in utero* exposure of the animals, lacked routine histological examinations of most tissues, the thorough gross pathology and subsequent microscopic examination of any grossly observed abnormalities would probably still have been adequate to detect carcinogenic effects. Almost all of the studies directed special attention to the urinary bladders, the site of the greatest concern about the carcinogenicity of cyclamate. Since cyclamate has been tested much more extensively than most compounds ever are, the deficiencies in one study may be compensated for by another study. The Cancer Assessment Committee of the Center for Food Safety and Applied Nutrition at the FDA recently addressed these problems and concluded that the collective evidence on cyclamate was adequate and that little would be gained by conducting additional standardized studies.[24]

J. Conclusion

None of the studies with cyclamate or cyclohexylamine conducted since 1970 have confirmed Oser's findings that implicated cyclamate as a bladder carcinogen. Furthermore, the recent animal studies have clearly demonstrated that cyclamate and cyclohexylamine are not carcinogenic in the urinary bladder or any other tissue. Similar conclusions have been reached by scientific and regulatory committees throughout the world as they have completed their reviews of the studies with cyclamate. For example, the Joint FAO/WHO Expert Committee on Food Additives[26] stated, "It is now possible to conclude that cyclamate has been demonstrated to be noncarcinogenic in a variety of species." The United States National Cancer Institute Committee for the Review of the Data on Cyclamate,[15] the Food Additives and Contaminant Committee of Great Britain,[225] and most recently the Cancer Assessment Committee of the Center for Food Safety and Applied Nutrition at the FDA[24] have all concurred in finding that the experimental data do not demonstrate cyclamate or cyclohexylamine to be carcinogenic. Although the NAS-NRC Committee[25] also concluded that the weight of the experimental evidence did not indicate that cyclamate by itself was carcinogenic, that group raised a question about possible promotional or cocarcinogenic activity, based primarily on the rat study by Hicks et al. and the mouse study by Bryan et al.

K. Human Studies

The possible association between the consumption of artificial sweeteners and cancer, particularly bladder cancer, in man has been extensively studied in the past 10 to 15 years. It is often difficult to separate any possible effects from saccharin and cyclamate because the two sweeteners were frequently used in combination. Since the widespread use of cyclamate in foods and beverages was restricted to a relatively short time span in many countries, the studies are probably more applicable to the assessment of the possible carcinogenicity of saccharin than cyclamate. However, at least five studies specifically addressed the cyclamate question or directed special attention toward subjects probably exposed to cyclamate during the 1960s.[257-261]

Four types of studies are included in this data base: (1) isolated case reports of bladder cancer in persons ingesting large amounts of the artificial sweeteners;[262] (2) trends in bladder cancer mortality over time;[263-264] (3) cohort studies of people, such as diabetics, who frequently use artificial sweeteners;[265-269] and (4) case-control studies in which persons with bladder cancer and their matched controls are studied with respect to their use of artificial sweeteners.[257-261,270-284] These studies have been reviewed by others[15,25,27,84,285-288] and will not be discussed in detail here. It has generally been agreed that the epidemiology studies do not provide conclusive evidence of an increased risk of bladder cancer associated with the use of the artificial sweeteners. The sensitivity of these studies has been questioned by some, and it is always possible that a very small increase in risk might not be detected. However, the studies with the artificial sweeteners are very extensive, involving, as Morgan[288] estimated, over 7,500 cases in the case-control studies and over 234,000 person years in the cohort studies. Whether additional studies would be helpful is debatable, although low-risk groups, *in utero* exposed individuals, heavy or long-term users, and those exposed many years earlier have been suggested for further assessment.[287-288,25]

VI. GENOTOXICITY

A. Gene Mutation Tests in Microbial and Mammalian Test Systems

Probably the most widely used gene mutation system is the Ames test, which detects reverse mutations in histidine-dependent strains of *Salmonella typhimurium*. McCann[289] of Ames' laboratory initially tested cyclamate and cyclohexylamine at concentrations ranging from 10 to 10,000 mcg/plate, both with and without a microsomal activation system from Arochlor-induced rat livers. Four tester strains (TA-100, TA-1535, TA-1537, and TA-98) were used so that both frame shift and base pair mutations could be detected. No significant increase in the reversion frequency was observed in any of the strains with either compound, although cyclohexylamine was inhibitory at the highest concentration. Subsequently, the negative results with cyclamate and cyclohexylamine in the Ames test have been confirmed by several different laboratories (Table 14).[290-296]

Neither compound has been studied as extensively in any other gene mutation system (Table 14), but the available information also suggests that cyclamate and cyclohexylamine are not mutagenic in these tests. Voogd (quoted in Cattanach[298]) performed a series of fluctuation tests for streptomycin resistance in *Klebsiella, Citrobacter, Enterobacter, Salmonella,* and *E. coli*. Cyclamate was not active, and cyclohexylamine caused a slight increase in the mutation frequency only in *E. coli* and only at an exceedingly high concentration (7500 mcg/mℓ). Neither compound was active when given to mice in host-mediated assays with these organisms. Negative results were also obtained with cyclamate and cyclohexylamine in other mouse host-mediated tests using *Salmonella* and *Serratia*.[297,299] The results with cyclohexylamine and cyclamate in the *E. coli* pol A^+/pol A^- test have been negative or inconclusive,[300-301] and Mayer et al.[302] reported that cyclohexylamine was inactive in an *E. coli* phage induction test. The only positive results in any microorganism test were Legator's[297] preliminary and unpublished findings that cyclohexylamine increased the mutation frequency in *Saccharomyces cerevisiae*.

Chu and Bailiff[303] reported the only in vitro test for gene mutations in a mammalian cell system. Chinese hamster cells were exposed to cyclohexylamine or *N*-hydroxycyclohexylamine for 24 hr and then evaluated for 8-azaguanine resistance. There was no increase in the mutation frequency in the cells treated with cyclohexylamine. *N*-Hydroxycyclohexylamine did, however, cause an increase in mutations, but only at concentrations that reduced cell survival to less than 20%.

B. Drosophila Studies

Cyclamate and cyclohexylamine have been tested in Drosophila by at least 11 groups of

Table 14
GENE MUTATION STUDIES WITH CYCLAMATE AND CYCLOHEXYLAMINE IN MICROBIAL AND MAMMALIAN TEST SYSTEMS

Test	Organism	Compound	Concentration	Results	Ref.
Ames test	*S. typhimurium* (TA-100, TA-1535, TA-1537, TA-98)	Cyclamate	10—10,000 mcg/plate	—	289
		Cyclohexylamine	10—1000 mcg/plate	—	
Ames test	*S. typhimurium* (TA-1535, TA-1538, TA-98, TA-100)	Cyclohexylamine	4-2500 mcg/plate	—	290
Ames test	*S. typhimurium* (TA-1535, TA-100, TA-1537, TA-98)	Cyclamate	12,500 mcg/plate	—	291—293
		Cyclohexylamine	2500 mcg/plate	—	
Ames test	*S. typhimurium* (TA-100, TA-98, TA-1537)	Ca Cyclamate	0.05—500 mcg/plate	—	294, 295
Ames test	*S. typhimurium* (TA-1535)	Cyclohexylamine	15 mcg	—	296
—	*S. typhimurium*	Ca Cyclamate	—	—	297
		Cyclohexylamine	—	—	
Host-mediated in mouse	*S. typhimurium*	Ca Cyclamate	—	—	297
		Cyclohexylamine	—	—	
—	*Saccharomyces cerevisiae*	Cyclohexylamine	0.05—0.3 *M*	+	297
Fluctuation test (streptomycin resistance)	*Klebsiella*	Na Cyclamate	—	—	298
	Citrobacter			—	
	Enterobacter			—	
	Salmonella			—	
	E. coli			—	
Fluctuation test (streptomycin resistance)	*Klebsiella*	Cyclohexlamine	—	—	298
	Citrobacter			—	
	Enterobacter			—	
	Salmonella			—	
	E. coli			+	

	Salmonella			—	
	E. coli			—	
Host-mediated	*Salmonella*	Na Cyclamate and	500 mg/kg, sc	—	299
in mouse	*Serratia*	Cyclohexylamine	100 mg/kg, sc	—	
Pol A^+/Pol A^-	*E. coli*	Cyclohexylamine	50 mcg/well	—	300
Pol A^+/Pol A^-	*E. coli*	Na Cyclamate	—	Inconclusive*	301
Phage Induction	*E. coli*	Cyclohexylamine	—	—	302
8-Azaguanine	Chinese hamster	Cyclohexylamine	1000 mcg/mℓ	—	303

Note: * No Inhibition of growth of either strain.

investigators (Table 15). Unfortunately, most of these studies have only been reported in abstract form, and the available data are frequently not sufficient for proper evaluation. The two studies published in most detail were both negative. Vogel and Chandler[304] found that the incidence of sex-linked recessive lethals was not increased in three broods of flies produced after feeding adult males with sodium cyclamate or cyclohexylamine. Knaap et al.[313] treated males with cyclohexylamine and *N*-hydroxycyclohexylamine either by adult injection or larval feeding and examined broods derived from both pre- and postmeiotic cells for sex-linked lethals (complete or mosaics) and II to III translocations. Their data provided no evidence of any mutagenic effect from cyclohexylamine or *N*-hydroxycyclohexylamine treatment. Browning[314] also reported negative results in a similar test with cyclohexylamine administered by adult injection.

In contrast to the uniformly negative findings with cyclohexylamine, the cyclamate studies present a conflicting picture. For example, Stith et al.[305] found an increased incidence of sex-linked lethals after feeding calcium or sodium cyclamate to larvae, but this could not be confirmed by Vogel and Chandler,[304] Rotter and Mittler,[307] or Moon et al.[308] Majumdar and Freedman[306] reported positive results for sex-linked lethals using the Muller-5 technique while Scram and Ondrel[310] reported negative results with the same test procedure. It, however, must be emphasized that most of these studies cannot be critically evaluated due to the incomplete presentation of the data. Hence, the results with cyclamate do not provide convincing evidence of a mutagenic effect, but neither do they alone support a conclusion of nonmutagenicity. Additional tests with cyclamate and cyclohexylamine in Drosophila are currently being conducted.

C. In Vitro Cytogenetic Studies

Sax and Sax[316] were the first to report chromosome aberrations in cells exposed to cyclamate in vitro, but their work involved onion root tips rather than a mammalian test system. In 1969, Stone et al.[317] found that high concentrations of sodium or calcium cyclamate (200 to 500 mcg/mℓ) increased the incidence of cells with chromosome breaks in human leukocyte or monolayer cell (human skin or laryngeal carcinoma) cultures. Subsequently, cyclamate and its major metabolite cyclohexylamine have been evaluated in a variety of in vitro test systems, as summarized in Table 16. Most of the studies used human leukocyte cultures stimulated with phytohaemagglutinin, but Chinese hamster fibroblasts, kangaroo rat kidney cells, and Chinese hamster lung cells have also been employed. Unfortunately, several of the studies were not reported in detail, thus making evaluation of the results more difficult. However, many, but not all, of the studies indicated that cyclamate and cyclohexylamine may cause a small increase in the frequency of chromosome gaps and/or breaks. The effects generally appeared to be dose-dependent,[178,317-320,322,327] but changes in the duration of treatment had variable effects. Some studies found that increasing the length of exposure did not affect the incidence of chromosome gaps and/or breaks,[318,328] others reported greater effects with longer treatments,[323,329] and yet another reported a delayed effect.[327]

In spite of the increased incidence of gaps and breaks, there is no evidence to suggest that cyclamate or cyclohexylamine treatment caused exchange figures, translocations, or other severe chromosome aberrations which are usually considered to be the best indication of true mutagenic effects. Gaps and breaks, similar to those seen with cyclamate and cyclohexylamine, may result from nonspecific toxicity and hence may not be indicative of real genetic damage.[298] Meisner and Inhorn[326] reasoned that if the chromatid breaks caused by cyclamate were true mutational events, they should lead to chromosome rearrangements in subsequent cell divisions and should persist after the compound was removed from the culture. On the other hand, if the breaks were due to nonspecific cytotoxicity, there should not be any persistent changes and the breaks should be diminished by removing the compound from the medium. Rearrangements were not seen in their fibroblast cultures even after

Compound	Concentration	Route	Parameters	Results	Ref.
Na Cyclamate	1—5 mg/mℓ	Adult feeding	Sex-linked lethals	−	304
Na + Ca Cyclamate	—	Larval feeding	Sex-linked lethals	+	305
			Salivary gland chromosomes	−	
Ca Cyclamate	1—5%	Larval feeding	Sex-linked lethals (Muller-5)	+	306
Ca Cyclamate	0.1—5%	Larval feeding	Sex-linked lethals		307
			Translocations	−	
			Chromosome loss, and Nondisjunction		
Ca + Na Cyclamate	5%	—	Sex-linked lethals	−	308
Cyclamate Acid	5%	—	Sex-linked lethals	±	
Ca Cyclamate	0.28—1%	Feeding	Chromosome exchange	+	309
			Nondisjunction	−	
Na Cyclamate	1%	Adult injection	Sex-linked lethals (Muller-5)	−	310
Na Cyclamate	0.05—1.60 mg/mℓ	Adult feeding	X-loss and nondisjunction	−	311
	10—100 mg/mℓ	Adult and larval feeding	X-loss and nondisjunction	+	
	50—160 mg/mℓ	Adult feeding	X-loss and nondisjunction	−	
Na Cyclamate	5%	Larval feeding	Crossing over -X chromosome	+	312
Cyclohexylamine	0.1—5 mg/mℓ	Adult injection	Sex-linked lethals,	−	313
	0.1—0.2%	Larval feeding	II-III translocations, and mosaic lethals		
Cyclohexylamine	0.01—1.0%	Adult injection	Sex-linked lethals and II-III translocations	−	314
Cyclohexylamine	1 mg/mℓ	Adult feeding	Sex-linked lethals	−	304
Cyclohexylamine	0.08—0.86 mg/mℓ	Adult and larval feeding	X-loss and non-disjunction		315

Table 16
IN VITRO CYTOGENETIC STUDIES WITH CYCLAMATE AN CYCLOHEXYLAMINE

Compound	Concentration Mcg/mℓ	Duration of exposure	Cell system	Resul
Na and Ca Cyclamate	50—500	72—84 hr	Human leukocytes	+
	200	5—6 days	Monolayer cultures	+
Na Cyclamate	2—200	5—25 hr	Human leukocytes	+
Na Cyclamate	1—500 mg	72 hr	Human leukocytes	+
Na Cyclamate	10—80	48 hr	Human leukocytes	+
Na Cyclamate	20	15 hr	Human leukocytes	–
Na Cyclamate	900—9000	—	Human leukocytes	+
Na Cyclamate	2000	3—6 days	Human leukocytes	+
Ca Cyclamate	20—4000	3 days	Human leukocytes	+
Na Cyclamate	500	3 days	Human fibroblasts	+
Ca Cyclamate	100—200	24 hr	Kangaroo rat kidney	–
Na Cyclamate	100—1000	1—3 days	Chinese hamster lung	+
Na and Ca Cyclamate	10—1000	3—124 days	Chinese hamster fibroblasts	+
Cyclohexylamine	1—100	5—25 hr	Human leukocytes	+
Cyclohexylamine	1—500	24 hr	Human leukocytes	+
Cyclohexylamine	20—500	15 hr	Human leukocytes	–
Cyclohexylamine	1—500	24 hr	Kangaroo rat kidney	+
Cyclohexylamine	10—1000	3—124 days	Chinese hamster fibroblasts	+

extended times, and hence the action of cyclamate was considered to be consis nonspecific cytotoxicity. Other investigators have also failed to observe chromoso locations and exchanges attributable to cyclamate or cyclohexylamine even thoug range of treatment times and conditions were used. However, several studies have that high concentrations of cyclamate and cyclohexylamine were cytotoxic and t slow cell division.[303,320,329,332-336] Moreover, the concentrations used in the in vitr were quite high, often reaching 1000 mcg/mℓ or more with cyclamate and 500 mcg cyclohexylamine. For comparison, the peak plasma levels found after a 5 g dose o cyclamate were about 20 mcg/mℓ[337] while a 10 mg/kg dose of cyclohexylamine g plasma concentrations of around 3 mcg/mℓ.[146] Hence, the concentrations routinel the in vitro studies were far in excess of the maximal levels achieved in man. In the contradictory nature of the results, the type of abnormalities observed, and concentrations used, the in vitro studies do not provide strong evidence that cyclar cyclohexylamine are genotoxic agents.

D. In Vivo Cytogenetic Studies with Mammalian Somatic Cells

Legator et al.[338] reported the first in vivo study showing chromosome aberrati the administration of cyclohexylamine (Table 17). Rats were given five daily intrap injections of cyclohexylamine base in doses ranging from 1 to 50 mg/kg. The pe of bone marrow cells with chromatid breaks was increased in a dose-related manne from 2.7% in the controls to 16.3% at 50 mg/kg. Exchange figures were observed infr and were not increased by cyclohexylamine treatment.

Using a similar experimental design, Dick et al.[339] were, however, unable to Legator's results. In their study, male rats of the same strain were again given fi 50 mg/kg doses of cyclohexylamine (as the base or hydrochloride) either orally peritoneally, and femoral bone marrow cells were examined for chromosome abnor The average percentage of cells with gaps or breaks was 2.1% in the rats given c

Table 17
CYTOGENETIC STUDIES WITH SOMATIC CELLS FROM ANIMALS TREATED WITH CYCLAMATE OR CYCLOHEXYLAMINE

Compound	Dose	Route	Duration	Species	Cell system	Results	Ref.
Ca Cyclamate	1%	Food	75 weeks	Rat	Bone marrow	–	54
Na Cyclamate	5%	Food	2—6 months	Rat	Bone marrow	+	319 178
Ca Cyclamate	10—100 mg/kg	Ip	5 days	Mongolian gerbil	Bone marrow	+	346, 347
Na Cyclamate — Saccharin (1:1)	4.8—10.2 mg/kg	Water	30, 60, 90 days	Rabbit	Leukocytes bone marrow	– –	348
Na or Ca Cyclamate	2—5 g	Po	300—1160 days	Human	Leukocytes	+	349
Na Cyclamate	4—5 g	Po	4 days	Human	Leukocytes	–	339
Na Cyclamate	3—16 g	Po	Up to 7 months	Human	Leukocytes	–	82
Cyclohexylamine (as base)	1—50 mg/kg	Ip	5 days	Rat	Bone marrow	+	338
Cyclohexylamine (as base and HCl)	50 mg/kg	Po, ip	5 days	Rat	Bone marrow	–	339
Cyclohexylamine (as HCl)	50—150 mg/kg	Food	Up to 18 months	Rat	Bone marrow	–	126, 340
Cyclohexylamine (as SO_4)	15—60 mg/kg	Po	>4 months	Rat	Bone marrow fetal kidney	– –	187
Cyclohexylamine (as base)	50—450 mg/kg	Ip	3 days	Chinese hamster	Bone marrow host-mediated with human leukocytes	– –	331, 341
Cyclohexylamine (as base)	200 mg/kg	Po	3 days	Chinese hamster	Leukocytes	+	342, 343
Cyclohexylamine (as base)	20—50 mg/kg	Ip	5 days/week for 7 weeks	Rat	Leukocytes	–	344
Cyclohexylamine (as base)	50—250 mg/kg	Iv	Single	Fetal lamb	Leukocytes	+	345

ylamine intraperitoneally, 2.4% after oral administration, and 3.2% in the control gr No reunion figures or fragmented metaphases were observed in either the treated or co groups. Mutagenic effects were obtained with the positive reference compound, thus d onstrating the validity of their test systems. Negative results were also found in bone ma cells from rats given cyclohexylamine sulfate orally in doses up to 89 mg/kg/day (~60 base/kg/day) for 4 months[187] and from rats given up to 150 mg base/kg/day in a 2 toxicity study.[126,340] Hence, the original findings of Legator et al.[338] must be questi since they could not be confirmed in three subsequent studies of bone marrow cells f rats receiving cyclohexylamine.

Brewen et al.[331,341] examined bone marrow cells from another species, the Chinese ham in a cytogenetic and host-mediated assay. Diffusion chambers containing human leukoc were placed in the peritoneal cavity of Chinese hamsters, which were then given three c intraperitoneal injections of cyclohexylamine at doses of 50, 150, or 450 mg/kg. The hu leukocytes and bone marrow cells from the host animals were examined for chromos abnormalities, but the percentages of chromatid breaks and achromatic lesions were significantly increased by cyclohexylamine treatment. About half the animals receiving 450 mg/kg dose died before the experiment was completed, but even this high dose still not cause clastogenic effects.

Three studies have examined leukocyte cultures prepared from cyclohexylamine-tre animals. Van Went-de Vries[342-343] found an increased incidence of chromosome abnormal in leukocyte cultures from Chinese hamsters given three daily oral doses of cyclohexylar (200 mg/kg). The structural aberrations included exchange figures, rings, breaks, and f ments, but no information about the incidence of the different types of abnormalities presented. Also, the percentage of abnormalities in the cultures prepared before cyclo ylamine treatment appeared to be unusually high. Mostardi et al.[344] gave rats cyclohexylar in doses of 20 or 50 mg/kg/day for 7 weeks and found no significant increase in the percen of cells with chromosome abnormalities. However, the control incidence was quite hig this study as well. Turner and Hutchinson[345] employed a novel technique to study the ef of cyclohexylamine on leukocyte cultures. Cyclohexylamine was administered to fetal la *in utero* at doses of about 50 to 250 mg/kg, and subsequently fetal blood samples v withdrawn for leukocyte cultures. Cyclohexylamine increased the incidence of both c matid breaks and major structural aberrations (e.g., rings, translocations, etc.), but was cytotoxic, as evidenced by a dose-related inhibition of cell growth.

Four studies have investigated the effects of cyclamate treatment on bone marrow or leukocyte cultures from laboratory animals. Freidman et al.[54] found that the freque of chromosome aberrations in bone marrow cells from male rats that had received 1% calc cyclamate in the diet for 75 weeks was well within the normal range for rats of that st and age. In contrast, Collin, Lederer, and their colleagues[178,319] reported chromosome normalities in bone marrow cells from female rats that had been given 5% sodium cyclar in their food for 2 to 6 months. However, the lack of any quantitative control data n evaluation of their findings impossible. Majumdar and Solomon[346-347] gave Monog gerbils five daily intraperitoneal injections of calcium cyclamate in doses of 10 to 100 kg/day and observed increased frequencies of hyperploid cells and chromatid breaks, g and fragments in the bone marrow cell preparations from the treated animals. The ef appeared to be dose-related up to, but not beyond the 30 mg/kg/day dose. In the stud Lisker and Cobo,[348] rabbits were given 5 or 10 mg/kg/day doses of a 1:1 mixture of sod cyclamate-saccharin for 90 days. No adverse effects were seen in either bone marrow or leukocyte cultures from the treated animals.

The most significant in vivo studies with cyclamate are those that looked for cytoge damage in man. Dick et al.[339] administered sodium cyclamate to four men and four wo in daily oral doses of 5 and 4 g, respectively, for 4 days. At least 100 metaphases v evaluated in the pre- and posttreatment leukocyte cultures of each subject. Urine anal

demonstrated that three of the subjects were converting cyclamate to cyclohexylamine during the study. The incidence of chromosome abnormalities (≤2%) in the leukocytes was not increased by cyclamate ingestion and was also similar to that found in an untreated control group. Gaps were the most common abnormality, and structural rearrangements or polyploidy were not seen. Wills et al.[82] also failed to observe any chromosome abnormalities attributable to cyclamate ingestion in their study with 32 prison volunteers, 24 of whom received cyclamate in daily doses ranging from 5 to 16 g. Most of their subjects ingesting cyclamate also excreted cyclohexylamine in the urine on one or more occasions during the study.

The third human study did, however, present evidence suggesting a possible clastogenic effect associated with the ingestion of cyclamate. Bauchinger et al.[349] administered sodium or calcium cyclamate to patients with liver or kidney diseases in doses of 2 to 5 g/day for 300 to 1160 days. A slight, but statistically significant, increase in the percentage of cells with chromosome aberrations (3.3%) was found in the leukocyte cultures from the patients ingesting cyclamate, as compared to the control groups of patients with similar diseases (1.4%) and healthy adults (1.3%). The most frequent type of aberration was chromatid breaks, but these effects did not appear to be correlated with either the cyclamate dose or the duration of treatment. The percentage of cells with chromosome translocations was increased to a similar extent in the patients receiving cyclamate (0.55%) and the control patients (0.52%), as compared to the normal subjects (0.07%), and hence could not be attributed to cyclamate. It is not possible to assess what effect, if any, the disease states, concurrent drug therapy, or diagnostic procedures may have had on the increased incidence of chromosome breaks in the patients receiving cyclamate since the two groups of patients could not be perfectly matched. Moreover, the reported incidence of chromosome abnormalities in the cyclamate group (3.3%) was still within the 0 to 4% range that is accepted as normal by many investigators.[331,339] Even the investigators concluded that, considering both the incidence and type of changes observed in the leukocyte cultures of the patients receiving cyclamate, it was rather unlikely that they had any medical significance.

E. Mammalian Germ Cell Studies

Of particular interest in assessing the risk of genetic damage from a compound are the tests involving mammalian germ cells (Table 18), since only mutations occurring in these cells can be transmitted to a subsequent generation. Somatic cell changes do not represent heritable genetic damage and are not necessarily indicative of the effects on germ cells.

Two studies have investigated the effects of cyclamate treatment on chromosomes from spermatogonia. Friedman et al.[54] gave rats diets containing 1% calcium cyclamate for 75 weeks and found that the incidence of chromatid breaks was similar in the treated and control groups. In the other study,[350] sodium cyclamate was administered orally to Chinese hamsters at a dose of 2000 mg/kg/day for 5 days. The percentage of cells with chromosome aberrations, either including or excluding gaps, was not significantly different from that in the control group, and no translocations were seen in the cyclamate-treated animals. Leonard and Linden[351-352] used an indirect method of examining spermatocytes for translocations induced in the spermatogonial stem cells. Mice were given sodium cyclamate in the drinking water to provide daily doses of about 400, 800, or 2000 mg/kg for up to 150 days. Examination of the spermatocytes revealed no evidence of any chromosome abnormalities attributable to the ingestion of cyclamate. Three studies have examined sperm from mice given five daily intraperitoneal injections of sodium or calcium cyclamate in doses up to 1000 to 2500 mg/kg/day, respectively.[294-295,353-354] The incidence of sperm abnormalities was not increased in any of these studies. Therefore, all the available information suggests that cyclamate does not induce heritable genetic damage in the germ cells of mice, rats, or Chinese hamsters.

Six studies have been performed on germ cells from animals treated with cyclohexylamine, and only one has reported positive results. Legator et al.[338] examined spermatogonial cells

Table 18
GERM CELL STUDIES WITH CYCLAMATE AND CYCLOHEXYLAMINE

Compound	Dose	Route	Duration	Species	Cell system	Results	Ref.
Ca Cyclamate	1%	Food	75 weeks	Rat	Spermatogonia	–	54
Na Cyclamate	2000 mg/kg	Po	5 days	Chinese hamster	Spermatogonia	–	350
Na Cyclamate	400—2000 mg/kg	Water	30, 60 or 150 days	Mouse	Spermatocytes for changes induced in spermatogonia	–	351, 352
Ca Cyclamate	60—500 mg/kg	Ip	5 days	Mouse	Sperm (1, 4 and 10 weeks after treatment)	–	353
Ca Cyclamate	300—2500 mg/kg	Ip	5 days	Mouse	Sperm (5 wk after treatment)	–	294
Na Cyclamate	100—1000 mg/kg	Ip	5 days	Mouse	Sperm (5 wk after treatment)	–	354
Na + Ca Cyclamate	26 + 3 mg/mℓ	In vitro	—	Chinese hamster	Ovary K-1 cells		355
Cyclohexylamine (as base)	1—50 mg/kg	Ip	5 days	Rat	Spermatogonia	+	338
Cyclohexylamine (as base or HCl)	50 mg/kg	Ip	5 days	Rat	Spermatogonia	–	356
Cyclohexylamine (as HCl)	50—150 mg/kg	Food	Up to 18 months	Rat	Testes	–	126, 340
Cyclohexylamine (as SO_4)	100 mg/kg	Po	5 days	Chinese hamster	Spermatogonia	–	357
Cyclohexylamine (as base)	50—100 mg/kg	Ip	5 days	Mouse	Spermatocytes for changes induced in spermatogonia	–	358
Cyclohexylamine	40—80 mg/kg	Ip	Single	Mouse	Spermatogonia and	–	177

Table 3
COLLINGS AND KIRKBY[122] STUDY: EFFECTS ON TESTES OF WISTAR RATS FED DIETS CONTAINING 0.01—1.0% CYCLOHEXYLAMINE HYDROCHLORIDE FOR 90 DAYS

Dose				Body weight (g)[b]		Food intake		Testes weight[d]		
	mg CHA/kg[a]									
% CHA·HCl in diet	Report	Calc.	N	Gain	Est. final	Total g	g/rat/day[c]	Abs. (g)	Rel.	Incidence of histopath. changes in testes[e]
0	0	0	16	240.9	320.9	1391.3	15.4	3.04	1.02	0/16
0.01	3.4	3.5	16	237.5	317.5	1385.1	15.4	2.94	1.01	0/16
0.05	18.5	17.6	16	236.6	316.6	1375.1	15.3	2.87	0.97	0/16
0.1	35	36	16	227.4	307.4	1375.1	15.3	2.89	1.03	0/16
0.2	116	69	16	220.6*	300.6	1280.9*	14.2	2.87	1.05	0/16
0.5	175	174	16	160.9*	240.9	1032.2*	11.5	2.65*	1.26*	0/16
1.0	434	352	15	94.3*	174.3	756.8*	8.4	0.96*	0.68*	13/15

Note: * Significant difference from control, $p \leq 0.05$.

[a] Reported values assumed to be CHA base/kg; calculated values determined using average food intake and estimated final body weight for males.

[b] Final body weight estimated from weight gain and assumed initial body weight of 80 g.

[c] g/rat/day calculated from total g/90 days.

[d] Weight of two testes; relative weight expressed as g/100 g body weight.

[e] Histopathological changes in testes represented bilateral degeneration of tubular epithelium. In 1.0% group, there was total degeneration in five rats, 95% in three rats, ≥70% in four, 40% in one and ≤1% in two.

tubules with luminal debris, or hydropic degeneration) was generally similar in the control and treated groups, although two rats in the 0.5% group exhibited some evidence of greater hydropic degeneration.

Gaunt et al.[123] at BIBRA gave groups of 15 male rats diets containing 0.06, 0.2, or 0.6% cyclohexylamine hydrochloride for 13 weeks. Additional groups of five rats received similar diets for 3 or 6 weeks or the 0.6% concentration for the entire 13 week period as part of a paired-feeding study. Body weight gain and food intake were significantly reduced at 0.2 and 0.6% in the diet, but the absolute and relative testicular weights were only decreased in the high dose group (Table 4). Initially, histological examination of the testes revealed a reduction in spermatogenesis and tubular atrophy in 4 of 11 rats at 0.2% and in 18 of 20 rats at 0.6%. However, in 1975 the original slides and freshly prepared slides were examined independently by two pathologists to better assess the incidence of the lesions.[163] The agreement between the two pathologists was reasonably good, and this second evaluation indicated that the incidence of the lesions was only increased in the rats receiving 0.6% cyclohexylamine hydrochloride in the diet (Table 4). However, rats treated with even the highest concentration for 10 months remained fertile in a small reproduction trial.[123]

The other study performed by Mason and Thompson[161] at BIBRA involved feeding groups of 25 male Wistar and Sprague-Dawley derived rats diets containing 0.06, 0.2, and 0.6% cyclohexylamine hydrochloride for 90 days (Table 5). Paired-fed and paired-weight control groups were included to assess the effects of decreased food consumption on the development of the testicular lesions. Body weight and food intake were depressed at 0.2 and 0.6% in both strains, compared to the *ad libitum* fed control groups. The weights of the high dose animals were also significantly lower than those of the paired-fed controls, but did not differ from the paired-weight groups. Testicular effects were only seen in the 0.6% groups, as exemplified by the reductions in the absolute weight of the testes, the sperm count and sperm motility, and histologically by an increased incidence of impaired spermatogenesis. In the affected animals, there was a marked reduction or the complete absence of spermatogenesis in many of the tubules. The only identifiable cell types remaining in these tubules were the Sertoli cells and a few spermatogonia; multinucleated cells were occasionally present. The basement membrane did not appear to be thickened nor were the Leydig cells involved. In contrast to these affected tubules, spermatogenesis appeared to be occurring in a normal fashion in other tubules. The absence of any effect in the paired-fed and paired-weight control groups clearly indicated that the testicular changes were not due to inanition, but were directly attributable to the highest concentration of cyclohexylamine.

Since these three studies had reasonably similar experimental designs, the results have been combined to provide an overall picture of the effects of cyclohexylamine on the rat testes (Figure 1). Body weight gain was not affected at dietary concentrations up to 0.1%. A slight decrease was seen at 0.2%, but pronounced, dose-related reductions occurred at the higher concentrations. Based on both the organ weight and histological changes, the testes did not appear to be affected at concentrations up to and including 0.2%, but clearly were at 0.6%. These concentrations would correspond to average doses of about 100 and 300 mg base/kg, respectively. Minimal, if any, effects were seen in the single group receiving 0.5%, which provided a dose of approximately 175 mg base/kg in that study.

In all of the above studies, cyclohexylamine was added to the diets of rats at fixed concentrations. This, of course, led to a progressive decrease in the milligram/kilogram dose that the rats ingested during the study. To circumvent this problem and to more precisely define the no-effect dose, a study designed at Abbott Laboratories was conducted by Brune et al.[162] Groups of 100 young male Sprague-Dawley rats were given diets providing daily doses of 50, 100, 200, or 300 mg cyclohexylamine base per kilogram. *Ad libitum* and paired-fed control groups were also included. At the end of the 3-month study, the body weights of the rats in all the cyclohexylamine treated groups were significantly decreased in com-

Table 4
GAUNT ET AL.[123] STUDY: EFFECTS ON TESTES OF CFE RATS FED DIETS CONTAINING 0.06, 0.2, OR 0.6% CYCLOHEXYLAMINE HYDROCHLORIDE FOR 90 DAYS

Dose										Incidence of histopathological changes in testes[d]							
	Avg. mg/kg		N[a]		Body weight (g)[b]			Testes weight[c]		Bilateral				Unilateral			
										All animals		13 wk trt.		All animals		13 wk trt.	
% CHA·HCl in Diet	CHA·HCl	CHA Base	13 wk	3—6 wk	Gain	Final	Food Intake g/Rat/Day	Abs. (g)	Rel.	No. 1[d]	No. 2[d]	No 1	No. 2	No. 1	No. 2	No. 1	No. 2
0	0	0	15	10	372	471	21.4	3.74	0.81	1/19	1/19	0/10	0/10	2/19	2/19	2/10	0/10
0.06	41	30	15	0	377	474	20.5	3.70	0.80	1/14	2/14	1/14	2/14	2/14	5/14	2/14	5/14
0.2	143	104	15	10	338*	435*	20.0*	3.43	0.81	0/22	2/22	0/13	2/13	5/22	3/22	4/13	3/13
0.6	468	342	15+5	10	274*	371*	18.6*	2.43*	0.67*	12/24	17/25	12/18	14/19	5/24	0/25	3/18	0/19

Note: * Significant difference from control, $p \leq 0.05$.

[a] Number of animals treated for 13 weeks and 3—6 weeks; five additional animals in 0.6% group were used in a 13 week paired feeding study.
[b] Body weight and weight gain data determined on day 84.
[c] Weight of two testes; relative weight expressed as g/100 g body weight.
[d] Histopathological assessments conducted in 1975 by two independent pathologists, designated as No. 1 and No. 2.

Table 5
MASON AND THOMPSON[161] STUDY: EFFECTS ON TESTES OF WISTAR AND SPRAGUE-DAWLEY RATS FED DIETS CONTAINING 0.06, 0.2, OR 0.6% CYCLOHEXYLAMINE HYDROCHLORIDE FOR 90 DAYS

	Dose			Body weight (g)[c]			Food intake		Testis weight[e]				Incidence of histopathological changes in testes[f]				
		Avg. mg/kg[b]							Absolute (g)		Relative						
Strain	% CHA · HCl in diet	CHA · HCl	CHA base	N	Gain	Final	Total g	g/Rat/Day[d]	L	R	L	R	N	1+	2+	3+	4+
Wistar	0	0	0	25	323	463	2047	22.7	1.71	1.66	0.38	0.37	25	0	0	0	0
	0—PF[a]	0	0	25	224*	365*	1430*	15.9	1.68	1.63	0.45	0.44	25	0	0	0	0
	0—PW[a]	0	0	25	182*	322*	1261*	14.0	1.57*	1.57	0.48	0.48	24	0	0	1	0
	0.06	46	34	25	314	455	2052	22.8	1.68	1.64	0.38	0.37	24	0	1	0	0
	0.2	149	109	25	269	409*	1841*	20.4	1.67	1.64	0.43	0.42	24	1	0	0	0
	0.6	416	304	25	181*+	322*+	1440*#	16.0	1.42*+#	1.40*+#	0.46	0.45	17	2[h]	2[h]	0	4
Sprague-Dawley	0	0	0	25	438	587	2391	26.6	1.89	1.78	0.33	0.32	24[g]	0	0	0	0
	0-PF	0	0	25	276*	426*	1686*	18.7	1.65*	1.70	0.40	0.41	25	0	0	0	0
	0-PW	0	0	25	254*	403*	1592*	17.7	1.67*	1.65	0.42	0.42	25	0	0	0	0
	0.06	44	32	25	422	570	2359	26.2	1.70	1.70	0.31	0.31	24	0	0	0	1
	0.2	140	102	25	380	531*	2149*	23.9	1.72	1.70	0.35	0.34	23	1[h]	1	0	0
	0.6	406	296	25	255*	405*	1687*#	18.7	1.39*+#	1.38*+#	0.36	0.36	17	1[h]	0	2	5

Note: * Significant difference from ad lib-fed control group, $p \leq 0.05$.
+ Significant difference from pair-fed control group, $p \leq 0.05$.
# Significant difference from pair-weight control group, $p \leq 0.05$.

[a] PF = pair fed with 0.6% group; PW = pair weight with 0.6% group.
[b] Average of 13 weekly determinations of CHA consumption.
[c] Body weight determinations at 13 weeks.
[d] Total food consumption per rat ÷ 90.
[e] Weight of testis; relative weight expressed as g/100 g body weight.
[f] Severity rating system based on percent of tubules affected: N = normal; 1+, <5%; 2+, 5—30%; 3+, 30—80%; 4+, >80%.
[g] Different type of lesion in left testis of one rat.
[h] Unilateral change in one rat.

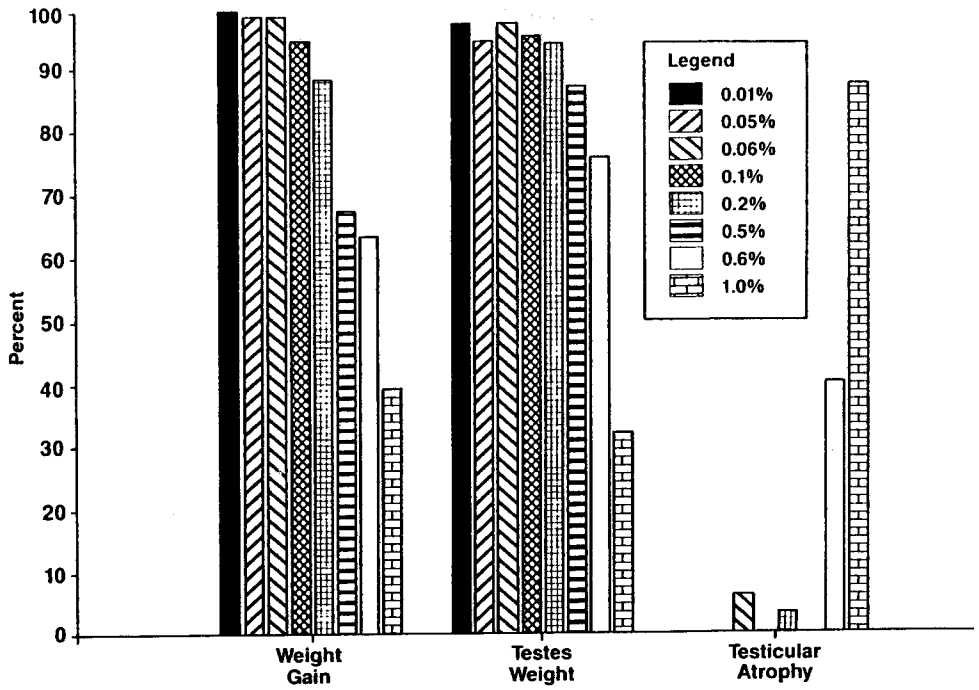

FIGURE 1. Effects of fixed dietary concentrations of cyclohexylamine hydrochloride on body weight gain the testes of rats in 3-month studies.

parison to the freely fed control group (Table 6). However, significant decreases in bo weight were only found in the 200 and 300 mg/kg/day groups when compared with th respective paired-fed control groups. The testicular weights were significantly lower in 200 and 300 mg/kg/day groups than the nontreated controls, but compared to the pair fed controls, a significant effect was only seen with the highest dose.

Three sections from each testes were examined microscopically for tubular alterati which were scored on a 0 to 4 scale. All slides were examined by a pathologist who v not aware of the treatment the animal had received. No differences in testicular scores w seen at 100 mg/kg/day, but the scores in the 200 and 300 mg/kg/day groups were significar higher than those of the *ad libitum* control groups and the corresponding paired-fed grou The increased testicular scores primarily resulted from a small number of animals that w severely affected, rather than from slight changes in a large number of rats. The most sev lesions consisted of degenerative changes in the tubules, giant cell formation, and compl testicular atrophy. In some cases only the Sertoli cells remained within the affected tubul Thus, this study demonstrated that 100 mg/kg/day was a no-adverse effect dose, and ba upon the slight changes at 200 mg/kg/day and marked effects at 300 mg/kg/day, sugges that the dose-response curve was quite steep.

The testicular effects of cyclohexylamine have also been evaluated in two chronic studi Gaunt et al.[124] found that the incidence of bilateral testicular atrophy (39%) was significar increased in the rats treated with 0.6%, but not 0.2, or 0.06%, cyclohexylamine hydrochlor in the diet for 2 years. The similarity of the effects in this 2-year study and the 90-(studies conducted in the same laboratory suggested that the lesions probably develo relatively early and did not become progressively more severe with continued treatment. another 2 year study, Oser et al.[125-126] observed a slightly higher incidence of testicu atrophy in the rats receiving the 50 and 150 mg/kg/day doses of cyclohexylamine than the controls or 100 mg/kg/day dose group. However, these findings were not regarded

Table 6
BRUNE ET AL.[163] STUDY: EFFECTS ON TESTES OF SPRAGUE-DAWLEY RATS FED DIETS CONTAINING CYCLOHEXYLAMINE HYDROCHLORIDE TO PROVIDE DOSES OF 50, 100, 200, OR 300 Mg CYCLOHEXYLAMINE PER KILOGRAM FOR AT LEAST 90 DAYS

	Treatment[a]			Body weight (g)[b]			Testes weight[d]				Histopathological changes in testes						
							Absolute (g)		Relative		Average score		Incidence of score				
Group	mg CHA/kg	Feeding	N	Gain	Final	Food Intake[c] g/Rat/Day	L	R	L	R	L	R	0—0.5	0.5—1.5	1.5—2.5	2.5—3.5	3.5—4
C_0	0	Ad lib	100	303	429	25.8	1.67	1.65	0.40	0.39	0.32	0.27	163	37	0	0	0
T_1	50	Ad lib	100	274	412 *	23.7	1.66	1.65	0.42	0.41	0.41	0.35	139	60	1	0	0
C_1	0	Pair fed	100	286	415	—	1.68	1.67	0.41	0.41	0.34	0.35[f]					
T_2	100	Ad lib	100	255	392*	22.3	1.65	1.63	0.44	0.43	0.40	0.35	137	63	0	0	0
C_2	0	Pair fed	100	266	398	—	1.68	1.66	0.43	0.42	0.34	0.38					
T_3	200	Ad lib	100	210	351*†	21.1	1.57*	1.55*	0.47	0.46	0.61*†	0.62*†	124	62	5	1	8*
C_3	0	Pair fed	100	227	367	—	1.62	1.61	0.45	0.44	0.35	0.32					
T_4	300	Ad lib	100	165	307*†	20.1	1.20*†	1.21*†	0.42	0.42	1.87*†	1.79*†	42	67	17	28	46*
C_4	0	Pair fed	100	212	344	—	1.64	1.63	0.48	0.48	0.42	0.46					

Note: *Significantly different from C_0, $p \leq 0.05$.
†Significantly different from paired control group, $p \leq 0.05$.

[a] Actual (determined) average mg/kg doses for T_1—T_4 groups were 50.2, 99.9, 198.8, and 297.4 mg CHA/kg.
[b] Body weight gain from day 3—112; final weight on day 105.
[c] Food intake represents an average of 15—17 determinations made at times ranging from day 3—105.
[d] Weight of one testis; relative weight expressed as g/100 g body weight determined just before sacrifice.
[e] Histopathological changes in testes evaluated in three sections of each testis and scored as: 0, no alterations; 1+, ≤5% of tubules affected; 2+, 6 to 20% affected; 3+, 21 to 60% affected; 4+, ≥61% affected. Each testis evaluated individually.
[f] Average scores of pair-fed groups estimated from average score of treated group and average difference.

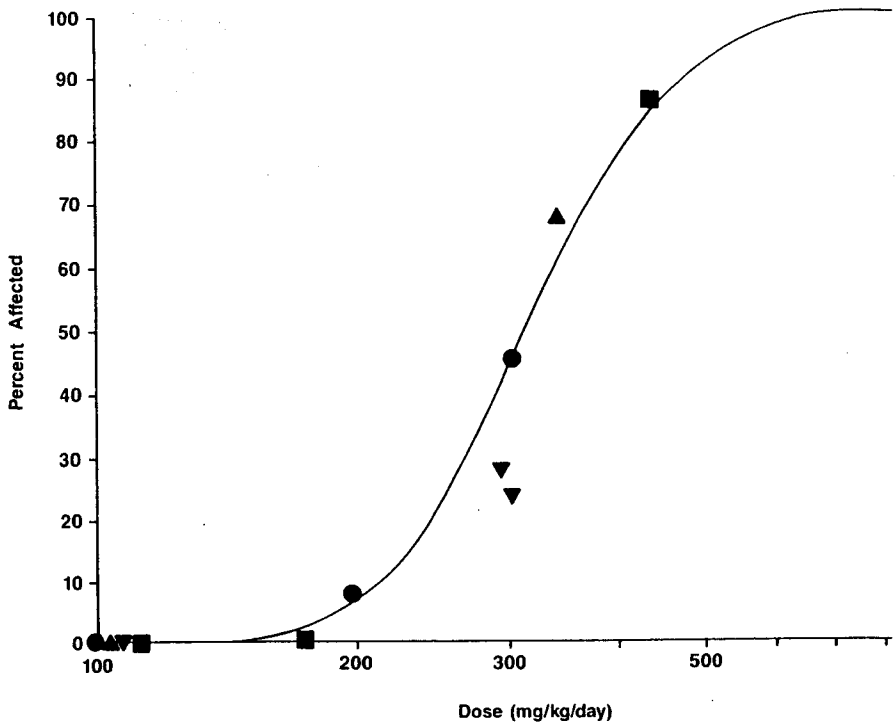

FIGURE 2. Dose-response curve for testicular atrophy induced by cyclohexylamine in rats during 3-mo studies. Symbols represent experimental observations in studies by Brune et al.[163] (●), Collings an (■), Gaunt et al.[123] (▲), and Mason and Thompson[161] (▼). Curve represents fitted probit model.

significant by the investigators, since the changes in the treated rats did not show dose-response relationship, the rats had remained fertile in the reproduction tests ticular atrophy is relatively common among older rats.

Since the testicular effects caused by cyclohexylamine in rats remain the toxi parameter that is probably the most sensitive to the effects of either cyclohexyl cyclamate, establishment of a no-adverse effect dose is critical to the evaluation of of the artificial sweetener. The work of Brune et al.[162] clearly showed 100 mg ba to be a no-effect dose. A slight, but appreciable, effect was seen at 200 mg ba while marked effects have occurred in numerous studies with doses in the 300 n range. Only two studies have investigated doses between 100 and 200 mg/kg/day — and Kirkby[122] whose 0.5% concentration corresponded to a dose of about 175 m and Oser et al.[125-126] who used 150 mg/kg/day in a 2-year study. Neither stud demonstrated adverse effects on the testes at these levels, but because of the smal of animals and the suggestion of possible effects, alone neither is adequate for cor establishing a no-effect dose. However, the steep nature of the dose-response curv 2) would be consistent with the no-effect dose being in the 150 to 175 mg/kg/da

Little is known about the mechanism of the cyclohexylamine-induced testicular rats. In spite of its sympathomimetic activity, cyclohexylamine did not increase the skin, or rectal temperatures of the rats.[122] Hence, the effects are probably not hyperthermia. Gaunt et al.[124] pointed out that arterial changes could be involv development of testicular atrophy. However, the incidence of cardiovascular le not increased in the cyclohexylamine treated rats, suggesting that if such a mechar involved it might be relatively specific to the blood vessels in the testes.

Gray and Beamand[164] have developed a mixed culture of Sertoli and germ cell testes to characterize the effects of phthalate esters and other testicular toxins

phthalate esters, which are thought to primarily exert their effects through the Sertoli cells, increased the rate of germ-cell detachment, but agents that acted directly on the germ cells did not. Cyclohexylamine, at concentrations of 10^{-4} or 10^{-3} *M* (10 to 100 mcg/mℓ) failed to increase germ-cell detachment, and the slight effect seen at 10^{-2} *M* (1000 mcg/mℓ) was accompanied by extensive cell death. The lack of a specific effect in this in vitro model and the persistence of the Sertoli cells in the tubules from rats treated with cyclohexylamine suggest that its primary effect is not on this cell population.

James et al.[165] found increased FSH and decreased testosterone levels in the serum of rats given 200 mg/kg/day doses of cyclohexylamine base by gavage for 9 weeks. Since these hormonal responses are characteristic reactions to a depletion of the germinal epithelium, they were considered secondary responses and not the primary cause of the testicular effects. There were no statistically significant effects on the weights of the testes, pituitary, prostate, or seminal vesicles, and the only lesion detectable by normal histological examination was focal atrophy of the seminiferous tubules in 1 of 15 rats. However, quantitative assessment of the stages of spermatogenesis indicated that cyclohexylamine treatment decreased the counts of pachytene spermatocytes and of early and late spermatids, without affecting the type B spermatogonia.

The effects of cyclohexylamine on the testes have not been as thoroughly evaluated in other species. Mice are clearly less sensitive and may be totally unaffected, as no adverse effects were seen in the testes of mice receiving 0.3% cyclohexylamine hydrochloride in the diet 80 weeks, equivalent to about 300 mg base/kg/day.[127] Cyclohexylamine does cause testicular effects in dogs, since James et al.[165] found decreases in the area of the testes and in the sperm count of the ejaculate from dogs given daily doses of 250 mg base/kg for up to 9 weeks. Quantitative assessment of the stages of spermatogenesis indicated that, as in rats, the pachytene spermatocytes and spermatids were primarily affected, but in contrast to rats, the effects in dogs were reversible during a 13-week recovery period. Neither the serum testosterone nor LH concentration was affected in the dogs. Since the dogs sometimes vomited after the administration of cyclohexylamine, the dose to which the animals were exposed may have been somewhat less than 250 mg/kg/day. The only other study in dogs gave no evidence of adverse effects on the testes of a few animals receiving cyclohexylamine sulfate in doses up to 150 mg/kg/day for over 9 years (approximately 100 mg base/kg/day).[121]

4. Central Nervous System

Acute toxic doses of cyclohexylamine in animals exert central stimulant effects, including hyperactivity, hyperexcitability, increased responsivity to external stimuli, and aggressive behavior.[37-38,43] Similar effects were observed in one subchronic toxicity study with rats given diets containing 0.5 to 1% cyclohexylamine hydrochloride,[122] but adverse behavioral effects were not seen in other rats ingesting diets containing up to 0.3 to 0.6% cyclohexylamine hydrochloride for 3 to 24 months.[123-126] Behavior was also apparently unaffected in mice given diets containing 0.3% cyclohexylamine hydrochloride in the diet for 80 weeks[127] and in dogs given up to 150 mg/kg/day doses of cyclohexylamine for several years.[121]

IV. TERATOGENIC AND REPRODUCTIVE EFFECTS

A. Cyclamate

1. Embryotoxicity

In 1964, Tanaka[166-167] reported that the oral administration of sodium cyclamate to pregnant mice on or before the 7th day of gestation was associated with a high degree of embryotoxicity. The fetal LD_{50} for cyclamate was estimated to be 180 mg/kg of the maternal body weight, but in addition to resorptions and late fetal deaths, fetuses showing retarded devel-

opment and judged to be nonviable were included in this calculation. Few impairments in development were observed when cyclamate was given on gestational days 8 to 10.

Other investigators subsequently attempted to confirm Tanaka's results, but none demonstrated a significant degree of embryotoxicity associated with the administration of cyclamate to mice or rats.[30] The numbers of resorption sites and viable young were not affected in mice given sodium or calcium cyclamate in doses up to 0.7 g/kg between the 3rd and 9th days of gestation. Similarly, no significant embryotoxic effects were observed in rats given calcium cyclamate in doses up to 2 g/kg on the fourth or seventh day of gestation.

Lorke[168] even used considerably higher doses of sodium cyclamate in an attempt to determine the fetal LD_{50}. Pregnant mice were given a single oral dose of 5 or 10 g/kg on the 5th, 7th, or 9th day of gestation. The fetuses were delivered by Caesarian section on the 18th day and thoroughly examined. The percentages of resorptions and dead fetuses ranged from 5 to 28% in the control groups, from 14 to 35% in the 5 g/kg group, and from 11 to 34% in the 10 g/kg groups. The percentages of underdeveloped fetuses ranged from 0 to 3% in the controls and 0 to 9% in the cyclamate treated groups. No significant differences were observed in the incidence of malformations and skeletal abnormalities. Even if all the fetuses which were either underdeveloped or malformed were included in estimating the LD_{50}, as Tanaka had done, the LD_{50} would still be greater than 10 g/kg. Since the oral LD_{50} of sodium cyclamate in adult mice is about 10 to 17 g/kg, there would appear to be little difference in the adult and fetal toxicity of cyclamate. Tanaka's data are also incompatible with the absence of effects in the six-generation study of Kroes et al.[60] in which mice were given up to 5% cyclamate in the diet (equivalent to about 7 g/kg/day).

2. Teratogenicity

The question of cyclamate teratogenicity was initially raised by the work of Verrett[169] in chick embryos. Calcium cyclamate or cyclohexylamine was introduced into the egg through the air cell either prior to incubation or during the period of rapid organogenesis (96 hr) at concentrations ranging from 0.05 to 200 ppm (200 ppm ~10 mg per egg). Both compounds caused an increased incidence of abnormalities, although cyclohexylamine was considerably more potent than cyclamate. The malformations involved the eyes (anophthalmia and microphthalmia), head (cleft palate and exencephaly), limbs (amelia, micromelia, phocomelia and syndactyly), and spine (spina bifida and curvature of the spine). Cyclamate-induced abnormalities in chick embryos were also reported by Ghiani and Muratori,[170] but not by Wolf et al.,[171] although relatively low concentrations were toxic to the developing embryo in the latter study.

Although cyclamate apparently can induce malformations in chick embryos, positive results in this avian test system are not considered indicative of a teratogenic potential in humans unless the findings are confirmed in mammalian tests. Numerous investigations in mice, rats, and rabbits have failed to demonstrate any significant increase in the incidence of malformations in the offspring of cyclamate-treated animals. Only the studies in which cyclamate was administered during the critical period of organogenesis, i.e., the typical phase II protocol, or those specifically dealing with teratogenic effects, are discussed in this section. In many other reproduction and embryotoxicity studies, the fetuses or neonates were examined for malformations, and negative results have consistently been reported.

Lorke,[172] Fritz and Hess,[173] and Klotzsche[174] employed similar protocols for studying the teratogenic potential of sodium cyclamate in mice, rats, and rabbits, respectively. Daily oral doses of sodium cyclamate (50, 100, or 250 mg/kg/day), sodium saccharin (5, 10, or 25 mg/kg/day), or sucrose (2, 4, or 10 g/kg/day) were administered to groups of 20 NMRI mice and Wistar rats on days 6 to 15 of gestation and to groups of 10 New Zealand rabbits on days 6 to 18. Two control groups were included in each study, one given tap water and the other left untreated. The dams were sacrificed on day 18 for the mice, day 21 for the

y 29 for the rabbits. The numbers of implantation and resorption sites, the litter
ie mean fetal weights were not adversely affected by any of the treatments in
three species. The types of malformations and minor skeletal changes, as well
lence, were also comparable in the test and control animals and gave no indication
ogenic effects of cyclamate.

conducted by Vogin, Oser, and their colleagues,[33-34,175] FDRL-Wistar derived
v Zealand rabbits were given a 10:1 sodium cyclamate-sodium saccharin mixture
500 or 2500 mg/kg/day on days 6 to 16 or 6 to 18 of gestation, respectively.
he number of implantation sites, number of live fetuses, and mean litter weights
in the control and treated groups. Only 1 malformed pup was observed in 23
dams given the 500 mg/kg dose, and no malformations were noted in the high
s. Examination of the developing skeleton revealed no treatment-related effects.
s, the administration of the cyclamate-saccharin mixture did not adversely affect
of implantation sites and live fetuses per litter, but the number of dead fetuses
be slightly greater in the treated animals. One fetus in the high dose group was
lformed. Skeletal examinations revealed no teratogenic effects, but suggested
tion might be delayed in the rabbits given the high dose. In light of the one
etus in the 2500 mg/kg group and the possible indication of fetal toxicity, the
epeated in rabbits given the high dose (2500 mg/kg). Although the pregnancy
in both the test and control groups, administration of the cyclamate-saccharin
no adverse effects on the numbers of implantation and resorption sites or the
live and dead fetuses. Examination of the fetuses and the developing skeleton
evidence of any abnormalities. No teratogenic effects were seen in other studies
given cyclamate orally in doses up to 1.0 g/kg[30] or rats given doses of 0.4 to
177

report of malformations related to the ingestion of cyclamate by rats was made
t al.[178] Wistar rats were fed diets containing 5% sodium cyclamate, and fetuses
arous females were examined on day 20 of gestation. Three cases of micro-
nd absence of the optic nerve were observed. Other fetuses reportedly had
s of the eyes, primarily involving fibrosis or vacuolization of the lens, but no
were included for comparison. Recently, Luckhaus and Machemer[179] attempted
ederer's finding of ocular abnormalities in cyclamate-treated rats. In their study,
eceived 5% sodium cyclamate in the diet for 20 days after mating. The fetuses
ıs were delivered by Caesarian section on day 20; other dams were allowed to
litters normally and the young were observed for 3 weeks. No cases of ano-
microphthalmia occurred, and histological changes were not seen in the lens,
y, optic nerve, or any other part of the eye. Postnatal eye function also provided
of ocular damage. Since no adverse effects were seen in this study, the authors
at the ocular changes described by Lederer may have represented spontaneous
s and/or histological artifacts.

oduction studies have been conducted in nonrodents. Derse[180] observed bilateral
he posterior limbs and cleft palates in two fetal pigs aborted from one sow fed
ning 5% sodium cyclamate. However, no teratogenic effects have been seen in
dogs receiving cyclamate. Pregnant rhesus monkeys were given 500 or 2000
of sodium cyclamate orally on 4 consecutive days between gestational days 20
ıe administration of cyclamate did not increase uterine deaths or malformations,
lence of any minor developmental variations was no greater in the cyclamate-
eys than the controls. Specific teratology studies have not been conducted in
malformations were seen in the offspring of dogs given the cyclamate-saccharin
to 1.5 g/kg/day).[46-47]

.[182] have developed an in vitro test for teratogens based on the differentiation
ɔ cells in culture. Pregnant rats were given the test compound intraperitoneally

on the 12th day of gestation, and 16 hr later midbrain and limb bud cells were taken for culture. After 5 days, growth, as determined by total protein, and differentiation, as determined by the incorporation of specific radiolabeled compounds, were assessed. Both parameters were depressed following exposure of the dams to the teratogens, but no effects were observed with the nonteratogens. Sodium cyclamate (500 mg/kg) was included in the battery of compounds used to validate this test and was identified as a nonteratogen. The sensitivity and specificity of the test exceeded 90%.

Mauer[183] exposed tubal stage rabbit embryos to a cyclamate-saccharin mixture (10:1) in vitro. Cleavage from the two-cell stage to the morula stage was not affected by concentrations up to 8000 mcg/mℓ. The embryos were subsequently implanted into the uterus of a rabbit and developed normally to near-term fetuses without any malformations.

3. Reproduction

a. Rats

Reproduction tests were conducted in rats as part of two chronic toxicity studies. A three-generation study in rats fed diets containing 1 to 3% sodium cyclamate revealed no significant differences in the fertility, gestation, and lactation indices or the litter size in any of the generations.[31] A one-generation, two-litter reproduction study was also performed in FDRL-Wistar derived rats fed diets containing a sodium cyclamate-sodium saccharin mixture (10:1) to provide daily doses of 500, 1120, or 2500 mg/kg.[33-34,175] In addition, other rats were treated with the low and high doses of the cyclamate-saccharin mixture from the 15th day of gestation through weaning. Again, there were no differences in the fertility, gestation, viability, or lactation indices of the control and treated rats. The numbers and body weights of the pups at birth and weaning were also similar in all treatment groups. Two other studies[50-51,176] which used lower doses of cyclamate also concluded that treatment with the artificial sweetener did not exert any adverse effects on the reproduction of rats.

In contrast to these studies, others have indicated that the growth and/or survival of the neonates may be impaired by high doses of cyclamate under certain circumstances.[52,64-65,71-72,180,184] Nees and Derse[64-65,180] fed rats diets containing calcium cyclamate (1, 5, or 10%) or sodium cyclamate (5 or 10%) either *ad libitum* or at 60% of that level. The rats on the limited food intake regimen, both the controls and those given 5 or 10% cyclamate, were not able to raise their first litters beyond 5 days. Similarly, the second litter pups in the control and 5% cyclamate groups died before weaning, while the dams receiving 10% cyclamate under the limited feeding conditions failed to conceive. The rats fed *ad libitum* were able to reproduce normally even with the high doses of cyclamate, but the weights of the animals at weaning were decreased by cyclamate in a dose-related manner. The average weights of the young on day 21 were 94% of the controls with 1% cyclamate in the diet, 80 to 89% with 5% and 62 to 68% with 10%. Although the body weights of the specific dams used in these reproduction studies were not reported, the 5 and 10% dietary concentrations generally caused 10 to 15% and 15 to 20% reductions in the body weights of the animals in this chronic toxicity study.

Ferrando and Hutchet[52] performed 3 generation reproduction studies in rats given 3% sodium cyclamate in the diet or 0.8 and 1.6% cyclamate in the drinking water. Neither the fertility nor resorption rate was affected by cyclamate treatment, and no malformations were observed. However, cyclamate administration decreased the survival and growth rate of the pups, especially in the second generation. The mortality rate in the control group was also higher than normal, which the authors suggested might be caused by the vitamin A deficiency in the diet or the advanced age of the females.

Zeman[184] followed the reproduction of rats fed diets containing 5% sodium cyclamate throughout gestation and lactation, and in addition to the usual untreated control group, a pair-fed group was included in this study. At birth some of the neonates were transferred

ns for the lactation period in an attempt to separate the effects of cyclamate
id lactation. The maternal weight gain was decreased to 59% of the controls
ite-fed rats and to 65% in the pair-fed animals. The percentage of females
and the number of young per litter were not affected by cyclamate or paired-
verage weight of the young at birth and their survival to weaning were
oth the cyclamate and pair-fed groups, but the effects on both parameters
reater in the cyclamate groups. The body weights at weaning were not reduced
g of the rats given cyclamate during pregnancy, but raised by control dams
n. However, the weights of the pups raised by dams receiving cyclamate or
lactation were similar to each other and lower than those raised by the control
the results of this study strongly suggested that the effects of cyclamate on
ie pups were secondary to the decreased food intake and its effect on lactation.
all the studies in rats suggests that cyclamate does not directly impair the
pacity of the rats. The only parameters that were even somewhat consistently
gh doses of cyclamate were the viability and growth of the pups from birth
ien these effects occurred, they were associated with reductions in the maternal
food intake, a vitamin deficiency, or aging, all of which might decrease
ed, Zeman's study clearly demonstrated that the effects of cyclamate on the
pups during lactation were similar to those produced by paired-feeding and
resulted from a reduction in the milk supply subsequent to the decreased
ion and body weight gain of the dams.

idies have investigated the effects of cyclamate on the reproduction of mice.
ttier-Arnould[185] reported an increased mortality rate and decreased growth in
f MNRI mice fed diets containing 5% sodium cyclamate. However, after an
eneration study Kroes et al.[60] concluded that cyclamate in concentrations up
dversely affect the reproduction of mice. In their study Swiss SPF mice were
itaining 2 or 5% sodium cyclamate or a 10:1 mixture of 2 or 5% sodium
0.2 or 0.5% saccharin. Some litters were followed through weaning and
idied *in utero* on day 20 of gestation. In addition, litters from the sixth
inimals receiving 5% cyclamate in the diet were thoroughly examined for
and ossification. Generally, the pregnancy rate, number of live fetuses, sex
rates on days 5 and 20, and the body weight on day 5 were not adversely
lamate treatment. The mean body weight of the pups at weaning was slightly
iree of eight litters from the mice receiving 5% dietary cyclamate. In the
s, no consistent effects from cyclamate treatment were seen on the number
sites, the number of living fetuses, the number of resorptions, or the mean
lo malformations attributable to cyclamate were observed in the teratology

i tests were performed as part of a 2-year toxicity study in dogs given
n cyclamate-sodium saccharin mixture at doses of 0.5, 1.0, and 1.5 g/kg/
ompounds were administered in the food for the first 14 weeks and then in
ig their first estrus period, four females from each group were mated with
same group, and then 5 to 6 months later the dogs were mated a second
emales had litters in both mating trials. The numbers of pups whelped and
average weights of the pups at birth and after 12 weeks were comparable

B. Cyclohexylamine

Initially, the standard three-phase reproduction studies were performed in rats given 1.5 or 15 mg/kg/day doses of cyclohexylamine sulfate orally, and it was concluded that these low doses of cyclohexylamine did not affect the reproduction of the animals.[186] Khera et al.[187] used somewhat higher daily doses of cyclohexylamine sulfate (22, 44, 89, or 178 mg/kg/day) in their rat reproduction study. The fertility of the females was not impaired, but male fertility appeared to be decreased in the first of three mating trials. No adverse effects were seen on embryo viability, litter size, litter weight, postnatal viability, or the weight gain of the pups. In another study, both male and female rats were given 0.2% cyclohexylamine sulfate in the drinking water, providing a daily dose of about 142 mg/kg.[188] After the first three mating trials, the males were given cyclohexylamine sulfate by gavage at a dose of 220 mg/kg/day while the females remained untreated. Male fertility, expressed as the number of females impregnated relative to the number exposed, and the total number of implantation sites were slightly decreased. The numbers of resorption sites, nonviable embryos, and malformed fetuses were similar in the control and test groups, excluding the possibility of a postimplantation embryocidal effect. Green et al.[189] also observed preimplantation losses in female rats mated with males that had been given 100 or 300 mg/kg doses of cyclohexylamine intraperitoneally. About 35% of the ova taken from the females did not show any evidence of cleavage, suggesting that fertilization had not occurred. In contrast to these studies, no impairment in fertility was seen in a small reproduction trial with males given 0.6% cyclohexylamine hydrochloride (~300 mg base/kg/day) in the diet for 10 months.[123]

Extensive reproduction studies were performed by Oser et al.[125-126] as part of their chronic toxicity study with rats receiving 15, 50, 100, or 150 mg/kg doses of cyclohexylamine in the feed. The parental generation (F_0) was mated to produce five litters, and rats from the first litter of each generation from F_0 through F_4 were mated to produce the next generation. Rats from the second litter of the F_1 through F_4 generations were also mated, with about half of the dams being used for teratology studies and the other half raising their young to maturity. The body weights of the dams, the size of the litters, and the weight of the pups at weaning were all slightly reduced with the higher doses (Table 7). A detailed statistical analysis of the data from this study was performed to determine if these effects were secondary to the decrements in the body weight of the dams.[14] Comparison of the results in the control and highest dose group (150 mg/kg) indicated that the reductions in the number of pups cast alive primarily occurred in the first litters of the different generations and that covariance analysis, with the dam weight at mating as the covariant, decreased or eliminated the statistical significance. The data for the pup weights on day 28 showed a more persistent effect through successive litters, but again covariance analysis usually reduced or eliminated the significance of the differences. Thus, this analysis strongly suggested that the effects on the number of pups born alive and the pup weight on day 28 were related to the decreased maternal weight, which in turn probably resulted from decreased consumption of the unpalatable diets.

Other females from this study were sacrificed prior to parturition, and the fetuses were examined *in utero*. The number of implantation sites, the number of live fetuses, and the incidence of malformations were not affected by cyclohexylamine treatment. Fetal weight appeared to be reduced with the highest dose in the F_1 generation, but was less affected in subsequent generations.

Kroes et al.[60] performed a six-generation reproduction study in mice given 0.5% cyclohexylamine sulfate in the diet. Growth retardation was seen in the mice receiving cyclohexylamine and was more pronounced in the females. Cyclohexylamine significantly decreased the number of live born fetuses, increased the postnatal mortality and decreased the body weight of the pups (Table 8). In the litters examined *in utero*, the number of implantation sites was reduced by cyclohexylamine, but no treatment-related malformations were seen.

Table 7
[OF CYCLOHEXYLAMINE IN FIVE GENERATION \T REPRODUCTION STUDY BY OSER ET AL.

Dam weight (g) first mating	Live pups per litter	Day 28 pup weight (g)	Pup survival (%)
260	10.7	81.9	97
264	10.5	83.6	98
247*	10.3	78.7*	99
231*	9.3*	75.6*	96
224*	8.0*	71.2*	96

cantly different from control ($P \leq 0.05$).

means of 124—195 litters. Data adapted from References 14, 125, and 126.

Table 8
' CYCLOHEXYLAMINE IN SIX GENERATION MOUSE EPRODUCTION STUDY BY KROES ET AL.[60]

oup	Pregnancy rate (%)	Pups cast alive	Postnatal survival		Body weight (g)	
			D5/D0	D20/D0	D5	D20
trol	90	10.7	58	37	2.3	8.5
\[b]	65*	9.2*	14*	10	0.8	7.1
trol	67	11.1	92	78	2.9	9.0
\	80	9.4	86	56*	2.1*	5.9*
trol	70	11.5	93	79	2.4	9.5
\	60	8.4*	75	49*	2.0	6.0*
trol	80	10.0	98	88	3.0	9.1
\	87	8.3*	80*	55*	2.4*	7.9
:rol	83	12.2	98	93	2.6	11.0
\	67	10.2*	87	76*	2.5	9.2*
:rol	75	11.1	99	85	3.2	11.3
\	83	9.3*	88*	75*	2.9*	9.2
rol	80	11.8	93	91	2.9	9.2
\	97*	10.5*	91	60*	2.5*	7.2*
rol	80	11.4	98	93	3.0	9.6
\	97*	9.9*	91	73*	2.5*	7.1*
rol	78	11.2	91	80	2.8	9.6
	80	9.4	76	57	2.2	7.4

lifferent from control $P \leq 0.05$.

tal generation bred to produce F_{1a} and $F_{1a'}$ generation. Further generations, des- n a straight line starting with the $F_{1a'}$ animals. In addition the F_{2a} animals produced gnated as F_{3b} which was used in a long-term toxicity study.
:lohexylamine sulfate in diet.

ported an increased mortality rate among the young of mice given 0.5 ne in the diet.

nich cyclohexylamine was only administered to the females during the sis have been performed. No malformations attributable to cyclohex- e seen in mice,[177,190-191] rats,[177,186] or rabbits.[186] However, an increased was reported in two studies involving intraperitoneal administration ase to mice (77 to 122 mg/kg)[190] and rats (10 mg).[160] In a recent study

by Lorke and Machemer,[192] cyclohexylamine hydrochloride was given orally by gavage at doses of 10, 30, or 100 mg base/kg/day to mice and rats on days 6 to 15 of gestation. Treatment with up to 100 mg/kg/day in mice and 30 mg/kg/day in rats had no adverse effects on the number of implantations, the resorption rate, sex ratio of the fetuses, fetal weight, placental weight, the incidence of malformations, and skeletal development. The only effects seen in the rats given the 100 mg/kg/day dose were reductions in the weights of the placenta and fetuses, but these changes were accompanied by decreases in the body weight gain of the dams. The authors, therefore, concluded that cyclohexylamine did not exert a teratogenic or primary toxic effect on the embryo and that the observed changes were secondary to the reductions in the maternal weight.

Wilson[181] gave rhesus monkeys four consecutive daily oral doses of cyclohexylamine (25, 50, or 75 mg/kg) between gestational days 20 and 45. Cyclohexylamine did not cause any increase in intrauterine deaths or malformations. There was some tendency toward lower fetal weights in the females treated earlier in the gestational period, but the small number of animals precluded any statistical analysis of the data.

Kitchin and Ebron[193] used an in vitro rat embryo culture technique to study the effects of cyclohexylamine. Concentrations of 0.1 and 0.3 m*M* (10 to 30 mcg/mℓ) had few adverse effects on the growth and differentiation of the rat embryo, but growth retardation and abnormal morphogenesis were seen at 1 m*M* or 100 mcg/mℓ. Assuming equal distribution throughout the body, these investigators considered the highest concentration to be equivalent to a 100 mg/kg dose and attributed the greater in vitro toxicity to the absence of the physiological excretory mechanisms.

Overall, it would appear that high doses of cyclohexylamine in rats and mice may be associated with adverse effects on reproduction, including decreases in the number of pups born alive, placental weight, fetal weight, pup survival, and pup growth. These changes were usually accompanied by reductions in the maternal weight and hence appeared to be secondary effects, dependent on the nutritional status of the females. None of the in vivo mammalian studies has given any indication of a teratogenic effect associated with the administration of cyclohexylamine.

V. CARCINOGENICITY

A. Introduction

Two of the first chronic toxicity and carcinogenicity studies with cyclamate were reported in the 1950s. Richards et al.[29] fed rats diets containing up to 1% sodium cyclamate for 18 to 24 months without observing any adverse effects attributable to the artificial sweetener. Fitzhugh et al.[53] gave Osborne-Mendel rats diets containing up to 5% sodium cyclamate for 2 years. Dietary levels of 1% or less were without effect, and the only effects seen at 5% were diarrhea and signs of mild inanition. Although all tissues, most notably the urinary bladder, were not examined microscopically, there was no evidence of a carcinogenic effect in either of these two studies.

With the increased use of a cyclamate-saccharin mixture in foods and beverages during the 1960s, additional toxicity tests were undertaken with this combination of sweeteners. The major issue concerning the safety of cyclamate arose when it was implicated as a bladder carcinogen in one of these studies. Oser et al.[33-34] fed groups of 35 male and 45 female rats diets containing the 10:1 mixture of sodium cyclamate and sodium saccharin to provide daily doses of 500, 1120, or 2500 mg/kg for 2 years. After 78 weeks, the diets fed to half of the rats were also supplemented with cyclohexylamine at levels corresponding to 10% conversion of the cyclamate dose (25, 56, and 125 mg cyclohexylamine per kilogram). Initially, eight bladder tumors were found in the high dose rats, with four to eight of the tumors being classified as carcinomas by the different pathologists who reviewed the slides.[9] In subsequent analyses, the number of tumors increased to 12 and all were considered carcinomas.[33]

ors were described as "nonmetastatic and for the most part noninfiltrating". e tumors, nonmalignant proliferative changes (e.g., epithelial hyperplasia vere found in the bladders of 6 control rats and 18 high dose animals.
dder tumors occurred in males and three in females. Five of the rats had ental cyclohexylamine, but seven had not. Three rats were classified as ., converted <0.1% of the daily cyclamate dose to cyclohexylamine), one er, and the rest were high converters (i.e., converted >0.7% of the daily cyclohexylamine). At necropsy, calculi were only found in the bladder with a tumor, but renal calcification was noted microscopically in six t had tumors. However, three high dose rats without tumors also showed reous deposits in the bladder. *Trichosomoides crassicauda,* a bladder id in five high dose rats, only one of which had a tumor. Hence, there ciation between the occurrence of the bladder tumors and either cyclo-ure, urinary tract calcification, or the presence of bladder parasites. Since ommon factors in the etiology of bladder tumors in rats,[194-198] a contribution m cannot be totally excluded. It has also been suggested that the devel-in six consecutively numbered and presumably consecutively housed male itive of a contribution from an extraneous environmental factor.[15,24] Al-as usually been focused on the urinary bladder tumors found in this study, ed out that complete histopathological examinations of the other organs t reveal any indication of a carcinogenic effect at any other site.
ons that contributed to the concern over the potential carcinogenicity of) included: (1) the occurrence of a bladder carcinoma in one male rat cylamine sulfate (15 mg/kg/day) in a 2-year toxicity study;[9,120] (2) the e of bladder tumors in mice that had cyclamate containing pellets implanted adders;[199] and (3) the discovery of bladder carcinomas in 3 of 23 rats cyclamate as part of a metabolism study.[54]
iment was one of two studies reported by Friedman et al.[54] Neither was inogenicity or even a chronic toxicity study, but histopathology exami-rmed on tissues from these rats when Oser's findings became known. In le Holtzman rats were given 1 or 2% calcium cyclamate in a semisynthetic equate (20%) or low (10%) protein levels. These rats were sacrificed after nent, and no carcinomas of the bladder were observed. A papilloma was ceiving 2% calcium cyclamate, but the kidneys and bladder of this animal nes or calcium deposits.
ly involved the treatment of male and female Osborne-Mendel rats with ing 0.4, 2.0, or 10% sodium or calcium cyclamate for 88 or 101 weeks. s occurred in one male and one female rat receiving 0.4% calcium cy-male receiving 10% calcium cyclamate. Both of these males had bladder tes were found in the kidneys of at least one rat with a tumor. Although provided additional circumstantial evidence of carcinogenicity, several t the three carcinomas found in this study may not have been attributable he incidence of the carcinomas was not dose-related or statistically sig-ree carcinomas were found in animals sacrificed at 88 weeks and no were found at 101 weeks; (3) there was a strong correlation between the adder calculi, and at least one rat with a tumor also had parasites in its 4) no carcinomas occurred in the rats receiving the same concentrations te in the chow diet or 1 to 2% calcium cyclamate in a semisynthetic diet. der papillomas were also reported[54] in three rats given sodium cyclamate calcium cyclamate, but these diagnoses could not be confirmed in a his study.[15]

Since 1970, cyclamate and cyclohexylamine have been reevaluated in a group of studies that were specifically designed to assess carcinogenicity and were performed by independent investigators throughout the world (Tables 9 and 10). Cyclamate was tested in at least five separate studies with rats, three studies with mice, and one study with hamsters. Both the sodium and calcium salts of cyclamate have been studied, and in addition the 10:1 cyclamate-saccharin mixture that was used by Oser has been tested three times in rats and once in mice. The studies included doses as high as 2.5 g/kg/day in rats, 7 to 9 g/kg/day in mice, and 3 g/kg/day in hamsters. Three experiments, two in rats[69,71-72] and one in mice[60] included *in utero* exposure of the animals. In addition to the conventional rodent bioassays, two studies have been conducted in monkeys treated with cyclamate for at least 8 to 12 years. Cyclohexylamine, as the hydrochloride or sulfate, was tested in three rat studies at doses up to 150 to 300 mg/kg and in two mouse studies at doses up to 400 to 600 mg/kg. A 9-year study was also conducted in dogs given daily doses of up to 150 mg cyclohexylamine sulfate per kilogram.

Each of these studies with cyclamate and cyclohexylamine will be briefly described, and then some of the issues raised during the evaluation of the results will be discussed. Based on the findings in Oser's study, the major question involved the development of urinary bladder tumors, particularly in rats. Therefore, special attention was directed toward the urinary bladders. In most of these studies, the bladders were inflated with a fixative, examined grossly, and then thorough histopathological examinations were performed. Subsequently, the possibility of an increased incidence of lung, liver, and/or lymphatic tumors in mice was raised as an issue in the 1980 decision on cyclamate by the commissioner of the U.S. FDA.[20] Hence, each of these topics will be discussed separately. Many of these studies have previously been reviewed in greater[15,20,23-25,27] and lesser detail[200-205] by others.

B. Studies with Cyclamate and Cyclamate — Saccharin Mixtures

1. Rats

a. Schmähl[68]

Groups of 52 male and 52 female Sprague-Dawley-derived rats were fed diets containing sodium cyclamate or a sodium cyclamate-sodium saccharin mixture (10:1) in concentrations of 2 or 5% for their lifetime. This strain of rat had previously been demonstrated to be sensitive to the bladder carcinogen, butylbutanolnitrosamine.[206] Histological examinations were performed on all bladders, but only on those other tissues that exhibited abnormalities at necropsy. No significant differences were observed in the incidences of or induction times for any tumors. Only one transitional cell carcinoma of the bladder was found. It occurred in a rat given 2% cyclamate and was accompanied by a bladder stone. Bladder parasites (Strongyloides and Capillaria) were detected in about 16% of the animals.

b. Schmähl and Habs[69]

A two generation study was also conducted by this same group of investigators. The parental generation of Sprague-Dawley rats was fed diets containing 2 or 5% of a sodium cyclamate-sodium saccharin (10:1) mixture for 3 months and then mated to produce the F_1 generation of rats. Groups of about 70 of these rats (males and females) were continued on the test diets for their lifetime. An additional group of rats received sugar (20% in the diet), and an untreated control group completed the experimental design. All urinary bladders and kidneys, as well as organs with macroscopically detected changes, were examined histologically. The distribution of the tumors did not differ significantly in the groups, and only one female rat in the 2% cyclamate-saccharin group developed a papilloma of the urinary bladder. However, stone formation was increased in the rats receiving 5% cyclamate-saccharin, and urinalysis results suggested that the levels of calcium oxalate, phosphates, and urates were also increased in the treated rats.

Table 9
CARCINOGENICITY STUDIES CONDUCTED WITH CYCLAMATE SINCE 1970

Study	Species	Strain	Sex	#/Group[b]	Compound	% Diet	Mg/kg[c]	Duration
Schmähl[68]	Rat	Sprague-Dawley	M+F	104	Na Cyclamate	2, 5	1000, 25000	Life
					Cyclamate/Saccharin (10:1)	2, 5	1000, 2500	
Schmähl[69]	Rat[a]	Sprague-Dawley	M+F	71—72	Cyclamate/Saccharin (10:1)	2, 5	1000, 2500	Life
Taylor[71-72]	Rat[a]	Sprague-Dawley	M+F	96	Ca Cyclamate	5	2500	Life
Ikeda[55-56]	Rat	Wistar	M	54—56	Na Cyclamate	5	2500	28 months
					Cyclamate/Saccharin (10:1)	5	2500	
Homburger[59]	Rat	Sprague-Dawley	M	50	Na Cyclamate	1, 5	500, 2500	24 months
Bar[44]	Rat	—	M+F	130, 30, 110	Na Cyclamate	—	150, 300, 450	2 years
Hicks[208-209]	Rat	Wistar	M+F	95—150	Na Cyclamate	2, 4	1000, 2000	2 years
Homburger[59]	Mouse	CD	M+F	100	Na Cyclamate	1, 5	1333, 6666	24 months
Brantom[45]	Mouse	ASH-CS1	M+F	60	Na Cyclamate	0.7, 1.75, 3.5, 7.0	933, 2333, 4666, 9333	80 weeks
Kroes[60]	Mouse[a]	Swiss-SPF	M+F	300	Na Cyclamate	2, 5	2666, 6666	21 months
					Cyclamate/Saccharin (10:1)	2.2, 5.5	2933, 7333	
Roe[67]	Mouse	Swiss	F	50	Na Cyclamate	5	6666	18 months
Rudali[210]	Mouse	C_3H; RIII; XVII/G; and $C_3H \times$ RIII	M+F	19—34	Na Cyclamate (in water)	0.6	1000	Life
Althoff[32]	Hamster	Syrian Golden	M+F	60	Na Cyclamate (in water)	0.156, 0.312, 0.625, 1.25	470, 1000 1900, 3800	Life
					Ca Cyclamate (in water)	0.156, 0.312, 0.625, 1.25	380, 800, 1400, 3110	Life
Coulston[48-49]	Monkey	Rhesus	M+F	5	Na Cyclamate	—	200	8 years
Sieber[70]	Monkey	Rhesus, Cyno, and Afr. Green	M+F	11—12	Na Cyclamate	—	100, 500	>12 years

[a] *In utero* exposure.

[b] Number of animals per group includes both males and females, where applicable.

[c] Mg/kg doses calculated from % in diet, assuming 20 g food/400 g rat or 4 g food/30 g mouse.

Table 10
CARCINOGENICITY STUDIES CONDUCTED WITH CYCLOHEXYLAMINE SINCE 1970

	Animals					Dose		
Study	Species	Strain	Sex	#/Group[a]	Compound	% Diet	Mg/kg[b]	Duration
Schmähl[68]	Rat	S-D	M+F	104	CHA	0.4	200	Life
Gaunt[124]	Rat	Wistar	M+F	96	CHA · HCl	0.06, 0.2 0.6	30, 100, 300	24 months
Oser[125-126]	Rat	Wistar	M+F	60	CHA (as HCl)	—	15, 50, 100, 150	24 months
Hardy[127]	Mouse	ASH-CS1	M+F	98	CHA · HCl	0.03, 0.1, 0.3	40, 133, 400	80 weeks
Kroes[60]	Mouse	Swiss-SPF	M+F	300	CHA · SO_4	0.5	666	21 months
Bio-Test[121]	Dog	Beagle	M+F	4	CHA · SO_4	—	0.15 → 50[c] 1.5 → 100 1/5 → 150	9.5 years

[a] Number of animals per group includes both males and females.
[b] Mg/kg doses calculated from % in diet, assuming 20 g food/400 g rat or 4 g food/30 g mouse.
[c] Doses increased after 3.7 years.

ther study by these same investigators,[207] female Sprague-Dawley rats were given e in doses of 0.2, 1, or 5 g/kg orally by gavage on days 14, 17, and 20 of their period. The offspring were observed throughout their lifetime, and no carcinogenic ıs detected.

***et al.*[55-56]**

ş of 54 to 56 male Wistar derived rats were given 5% sodium cyclamate or a e-saccharin mixture in their diet starting at 5 weeks of age and continuing for up nths. The bladders were examined microscopically, and no tumors were observed the test or control groups.

***- et al.*[71-72]**

ıp of 48 male and 48 female *in utero* exposed Charles River CD rats (Sprague- derived) were given a diet containing 5% calcium cyclamate for their lifetime nately 28 months). These animals were the offspring of parents fed the test diet ıning through mating, gestation, and lactation. The control group received sodium : (1.5%), and other groups were given sodium saccharin at concentrations ranging 1 to 7.5%. Histological examinations in the cyclamate group included the urinary liver, heart, kidneys, lungs, adrenals, bone marrow, and any grossly abnormal ıe incidences and types of tumors in the cyclamate-treated rats were not significantly from those in the controls. No bladder tumors were found in the cyclamate group, umor (transitional cell polyp) occurred in a control male rat.

***urger*[59]**

ı cyclamate was fed to duplicate groups of 25 male Charles River CD-1 rats -Dawley derived) at dietary levels of 1 and 5% (total of 50 rats per dose), while a 25 males served as the untreated controls. The animals were started on the test ɔout 8 weeks of age and continued for 24 months. Histological examinations were ɪ on the urinary bladders, all vital organs from at least 12 animals in each group, grossly abnormal organ. A noninvasive papillary carcinoma of the bladder was one control rat. In one of the test replicates, a bladder carcinoma occurred at the -y level, and one carcinoma *in situ* and one papilloma of the bladder were found :s given 5% cyclamate in the diet. No bladder tumors were observed at either .tion in the second replicate study. Thus, the overall incidence of bladder tumors ppear to be related to cyclamate treatment. Ova consistent with the presence of *noides crassicauda* were found in about one third of the urine samples examined, presence was not correlated to the bladder lesions.

***et al.*[208-209]**

t of a cocarcinogenicity study with *N*-methyl-*N*-nitrosourea, a group of 245 male le SPF Wistar rats were given diets containing 2 or 4% sodium cyclamate for 2 stological examination of the bladders from 228 rats revealed tumors in one rat 2% concentration (1 g/kg/day) and 2 rats given 4% cyclamate (2 g/kg/day). Even o bladder tumors were seen in a group of 105 untreated controls (98 bladders l microscopically), the incidence of the tumors in the treated groups was not sig- increased. The first tumor developed after 87 weeks of treatment. The two tumors ;h dose animals were transitional cell carcinomas, but the tumor in the low dose ıs classified as a spindle cell sarcoma (probably a leiomyosarcoma) by the NCI e[15] who reviewed the slides. Calculi were found in two of the rats with tumors, ş been reported that signs of mineralization were also noted in the other animals

***g. Bar and Griepentrog*[44]**

In a study for which very little information is available, groups of rats were given sodium cyclamate at doses of 150, 300, or 450 mg/kg/day for 2 years. No bladder tumors were seen in the low-dose group, but the results from the highest dose have apparently not been reported.

2. Mice

***a. Brantom et al.*[45]**

Groups of 30 male and 30 female ASH-CS1 mice were fed diets containing 0.7, 1.75, 3.5, or 7.0% sodium cyclamate for 80 weeks while a group of 60 mice of each sex served as the untreated controls. Relatively complete histopathological examinations were conducted in the control and high dose group, but at the intermediate levels, the microscopic examinations were confined to the urinary bladder, heart, liver, kidneys, and any other tissue that appeared abnormal at necropsy. No bladder tumors were observed in any of the mice. The most common tumors included lung adenomas, and in the reticulo-endothelial system, lymphosarcomas, and reticular cell sarcomas. The authors concluded that there were no effects attributable to cyclamate with respect to the incidence of any tumors or other histopathological changes.

***b. Homburger*[59]**

In two duplicate bioassays, sodium cyclamate was fed to groups of 25 male and 25 female Charles River CD mice at dietary concentrations of 1 and 5% (total of 50 mice per sex per dose) for 24 months. A group of 25 males and 25 females served as the untreated controls. Histological examinations were conducted on the urinary bladders, all vital organs from at least 12 animals per group, and any grossly abnormal tissue. One control male mouse developed a transitional cell carcinoma of the bladder which was accompanied by a large stone, but no bladder tumors were found in any of the cyclamate-treated mice. The incidence of other tumors did not appear to be affected by the administration of cyclamate.

***c. Kroes et al.*[60]**

In a multigeneration study, Swiss mice were given sodium cyclamate at dietary concentrations of 2 and 5%, a sodium cyclamate-saccharin mixture (10:1) at dietary concentrations of 2.2 and 5.5%, saccharin at concentrations of 0.2 and 0.5%, or cyclohexylamine sulfate at a dietary concentration of 0.5%. Groups of 50 males and 50 females from the parental generation and from two subsequent generations (F_{3b} and F_{6a}) were continued on these diets for up to 21 months. Histological examinations included the urinary bladder and most major organs, as well as any other tissue showing macroscopic changes. Seven bladder tumors were found in a total of 2400 animals, and each was detected in a different treatment group (i.e., an anaplastic carcinoma in a female control mouse of the P generation, a transitional cell carcinoma in a female of the F_{6a} generation given 5% sodium cyclamate, a papilloma in a male of the F_{6a} generation given 5% sodium cyclamate, a papilloma in a male of the F_{6a} generation given 2.2% cyclamate-saccharin, anaplastic carcinomas in one male of the P generation and one female of the F_{6a} generation given 2.2% cyclamate-saccharin, and two carcinomas in males receiving only saccharin). Hence, the distribution of these tumors, and other tumors as well, gave no indication of a carcinogenic effect from cyclamate.

***d. Roe et al.*[67]**

As part of a cocarcinogenicity study with benzo[a]pyrene, a group of 50 female Swiss mice was given food containing 5% sodium cyclamate for 18 months. Major organs including the urinary bladder were examined macroscopically but microscopic examination was restricted to the grossly observed lesions. No bladder tumors were found, and the incidence of other tumors did not appear to be affected by cyclamate treatment.

al.[210]

ıl.[210] gave mice sodium cyclamate in the drinking water at a concentration of ily consumption was estimated to be about 20 to 25 mg per mouse or less than reatment was continued for the animals' lifetime. The test groups included 9 female C_3H mice, 22 male RIII mice, 20 female XVII/G mice, and 34 F_1 males between C_3H and RIII mice. Untreated control groups of approximately the re maintained for all strains. Statistically signifiant increases in the percentages tumors were observed in the treated XVII/G females (80% vs. 19%) and the III) males (82% vs. 57%). Lung tumors were predominant in the former strain nors), whereas primarily liver (22 of 29 tumors) and a few lung tumors (7 of ere found in the latter.

al.[32]

30 male and 30 female Syrian golden hamsters were given sodium or calcium the drinking water at concentrations of 0, 0.156, 0.312, 0.625, and 1.25%, veeks of age and continuing throughout their lifetime. The daily consumptions : concentration averaged 380 and 311 mg with the sodium and calcium salts, and provided a dose of around 3 g/kg. The overall tumor incidences were groups, and the organ distribution and types of neoplasms were within the spontaneously occurring tumors in this strain of hamsters. No urinary bladder found, even though this species had been considered to be a good model for lasms.

et al.[48-49]

; monkeys were given sodium cyclamate orally at doses of 200 mg/kg/day, 6 for up to 8 years. When three animals which had been treated for over 90 sacrificed, no pathological changes were detected grossly in any organ or lly in the liver, kidneys, urinary bladder, or testes.

l Adamson[70]

ing study, groups of 11 or 12 male and female monkeys have been given mate orally in doses of 100 or 500 mg/kg/day, 5 days a week, for over 12 ıonkeys represented at least three species: rhesus, cynomolgus, and African has been no evidence of any carcinogenic effect associated with the admin- /clamate.[70,119]

ith Cyclohexylamine

52 male and 52 female Sprague-Dawley rats given 0.4% cyclohexylamine in valent to 200 mg/kg, was included in the original study by Schmähl[68] (see ..). No bladder tumors were found in these rats, and there was no indication enic effect from cyclohexylamine treatment.

l.[124]

48 male and 48 female Wistar rats were given diets containing 0.06, 0.2, and xylamine hydrochloride for 2 years. Histopathological examinations were con- st tissues, including the urinary bladder, from the animals in all groups. There ıce of a carcinogenic effect at any concentration of cyclohexylamine, and no s were observed.

c. Oser et al.[125-126]

Cyclohexylamine hydrochloride was fed to groups of 30 male and 30 female FDRL (Wistar derived) rats for 2 years to provide doses of 15, 50, 100, and 150 mg base/kg/day. Histological examinations were made on at least 20 organs from 15 to 20 rats of each sex in the control and highest dose group and on 8 major organs from 10 or more rats of each sex in the other groups. Extensive examinations of the urinary bladders revealed no tumors, and the incidences of other tumors were similar in all groups, including the controls.

2. Mice

a. Kroes et al.[60]

Cyclohexylamine sulfate (0.5% in the diet) was included in the extensive multigeneration mouse study conducted by Kroes et al.[60] (see Section B.2.c.). Cyclohexylamine did not show any carcinogenic effect, and no bladder tumors were found in the cyclohexylamine-treated mice.

b. Hardy et al.[127]

Groups of 48 male and 48 female ASH-CS1 mice were fed diets containing 0.03, 0.1, or 0.3% cyclohexylamine hydrochloride in the diet for 80 weeks. Relatively complete histopathological examinations were conducted on the tissues of the mice from all treatment groups. There were no statistically significant differences in the tumor incidences of the cyclohexylamine-treated and control groups, and no bladder tumors were found.

3. Dogs

a. Industrial Bio-test Laboratories[121]

A small chronic toxicity study was also conducted in dogs. Groups of 2 male and 2 female beagles were given cyclohexylamine sulfate in doses of 0.15, 1.5, and 15 mg/kg/day for almost 4 years, and then the doses were increased to 50, 100, and 150 mg/kg/day for an additional 6 years. No effects attributable to cyclohexylamine were seen in this study.

D. Bladder Tumors

The incidence of bladder tumors in these recent rodent carcinogenicity studies with cyclamate and cyclohexylamine is summarized in Table 11. A careful examination of these data indicates that the administration of cyclamate and cyclohexylamine did not cause tumors to develop in the urinary bladders of these animals. In no study since Oser's original experiment was there a statistically significant increase in the incidence of bladder neoplasms in the treated animals. Furthermore, the pattern of the few bladder tumors that were seen gives little credence to the thesis that they were caused by the test agents. Nevertheless, questions about the ability of cyclamate to induce bladder tumors have still been raised.[20,209,234] These have largely revolved around the possible biological significance of the few bladder tumors that did develop in the cyclamate treated rats since these tumors are "relatively rare". In an effort to overcome the difficulties of demonstrating statistical significance for tumors that occur in a low frequency, the commissioner of the FDA attempted to combine the results from all the doses of cyclamate in all the studies with the same strain of rats (i.e., Sprague-Dawley or Wistar) and then to compare the combined tumor incidence to historic rather than the concurrent controls.[20] Such techniques are generally considered inappropriate and are not usually accepted in evaluating the results from the carcinogenicity bioassays. Indeed, many aspects of the statistical analyses and interpretations presented in that decision have been strongly criticized by the American Statistical Association[21] and others.[22,24,211]

Considering the negative results in all of these studies, the high incidence of bladder tumors in Oser's study with the cyclamate-saccharin mixture remains inexplicable. One of

Table 11
SUMMARY OF BLADDER TUMORS IN RECENT RODENT CARCINOGENICITY STUDIES WITH CYCLAMATE, CYCLAMATE-SACCHARIN MIXTURES, AND CYCLOHEXYLAMINE

Incidence of Bladder Tumors (Tumors/Initial Group Size)

	Cyclamate % in Diet									Cyc-sac % in Diet		
Animal	**Controls**	**0.7**	**1.0**	**1.75**	**2.0**	**3.5**	**4.0**	**5.0**	**7.0**	**2 or 2.2**	**5 or 55.5**	**Ref.**
Sprague-Dawley rats	0/104*				1/104 (C; M)			0/104		0/104	0/104	68
	0/70									1/72 (P; F)	0/71	69
	1/25 (C; M)		1/50 (C; M)					2/50 (C,P; M)				59
	1/96 (T; M)							0/96				71, 72
Wistar rats	0/98				1/95 (S; M)		2/150 (C; M)					208—209
	0/54							0/56			0/54	55, 56
Mice (all strains)	0/120	0/60		0/60		0/60			0/60			45
	1/300* (C; F)				0/300			1/300 (C; F)		3/300 (P,2C;2M,F)	0/300	60
	1/50 (C; M)		0/100					0/100				59

	Cyclohexylamine % in Diet									
Animal	**Controls**	**0.03**	**0.06**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Ref.**
Rats (all strains)	0/96		0/96		0/96				0/96	124
	0/60	0/60		0/60	0/60	0/60				125, 126
	0/104*						0/104			68
Mice (all strains)	0/98	0/98		0/98		0/98				127
	1/300* (C; F)							0/300		60

Note: C = Carcinoma; P = Papilloma; S = Sarcoma; T = Transitional Cell Polyp; M = Male; F = Female

* Same control group for cyclamate and cyclohexylamine.

the basic tenets of the scientific process is that experimental results must be replicable. The role of cyclamate in causing bladder tumors has not been confirmed; instead, cyclamate has repeatedly been shown to be noncarcinogenic in rats and mice. Furthermore, the studies in hamsters, dogs, and monkeys support the conclusion that cyclamate and cyclohexylamine are not bladder carcinogens.

The results from the cyclamate and cyclohexylamine bioassays also suggest that, when bladders from experimental animals are examined meticulously, spontaneous tumors are detected and probably occur more frequently than originally thought.[24] This trend also makes the use of historical control data less appropriate, since similar techniques were not routinely used in examining the bladder in the past.

An association also appears to exist between the few tumors that did occur and the presence of calculi. For example, in Schmähl's study the one carcinoma that developed in a rat given 2% cyclamate was accompanied by large calculi. In Hicks' study, at least two of the bladder tumors that occurred in the rats receiving cyclamate were accompanied by stones. In Friedman's earlier study, two of the three tumors developed in rats which also had calculi. The presence of both calculi and tumors cannot prove a cause and effect relationship, and the correlation between the tumors and stones is not perfect (i.e., calculi were not found in all rats with bladder tumors and all rats with calculi did not develop tumors). However, it is well established that calculi frequently contribute to the development of bladder tumors in rodents.[194-197] Implantation of pellets or other foreign bodies in the bladder of mice or rats leads to hyperplasia of the urothelium and the development of tumors.[212-214] Furthermore, the bladder tumors induced by several chemicals, including 2,3′-azotoluene,[215] diethylstilbestrol,[216] diethyleneglycol,[217-219] polyoxyethylene-8-stearate,[220-221] 4-ethylsulfonylnaphthalene-1-sulfonamide,[222-223] and melamine,[224] have all been linked to the presence of calculi. Hence, Clayson[194] has cautioned that care must be exercised in classifying a chemical as a direct bladder carcinogen if it also provokes the formation of urinary calculi. In contrast to the situation in rodents, little or no relationship has been found between bladder stones and tumors in man.[194]

E. Tumors of the Lymphoreticular System

The issue of tumors in the lymphoreticular system of mice was raised in the 1980 decision of the commissioner of the FDA[20] and was based on the studies by Brantom et al.,[45] Kroes et al.[60] and Hardy et al.[127] Pertinent data from these studies are summarized in Table 12. Specifically, dose-related increased incidences of lymphosarcomas in the female mice from the Brantom study and in the male mice from the Kroes study were attributed to cyclamate. Although a statistically significant increase in the incidence of lymphosarcomas was not seen in Hardy's study with cyclohexylamine, the data were called supportive of the results with cyclamate. However, examination of the data reveals certain features that are difficult to reconcile with a treatment-related effect. In the Branton study, the incidence of lymphosarcomas in the females has been reported to be within the normal range for that strain of mice in that laboratory,[225] and no such tumors were found in the high dose males. In contrast to the first two generations of male mice in the Kroes study, the high dose males in the last generation had a lower incidence of lymphosarcomas alone or with leukemia than either the controls or low dose animals. No effect was seen in the females of this study. Also, no correlation was noted between the involvement of the lymph nodes and spleen, making classification of the lesion doubtful.[225]

Questions have been raised about the statistical techniques used in the 1980 decision,[20] such as the proper use of the Bonferroni multiplier and the Cochran-Armitage test. Moreover, some of the statistical analyses were performed on the combined incidences of lymphosarcomas and reticular cell sarcomas or leukemias and lymphosarcomas, and the results from the three generations in Kroes study were also pooled. It is generally considered inappropriate to combine the tumor incidences in different generations or the incidences of different types

Table 12

	Males					Females				
Sodium Cyclamate:	**0**	**0.7%**	**1.75%**	**3.5%**	**7.0%**	**0**	**0.7%**	**1.75%**	**3.5%**	**7.0%**
N	46	21	27	23	24	45	19	18	21	25
Lymphosarcomas	7 (15%)	2 (10%)	2 (7%)	2 (9%)	0 (0%)	3 (7%)	2 (11%)	3 (17%)	4 (19%)	6 (25%)
Reticular cell sarcomas	1 (2%)	1 (5%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)	2 (11%)	1 (6%)	1 (5%)	0 (0%)
Both	8 (17%)	3 (14%)	3 (11%)	2 (9%)	0 (0%)	3 (7%)	4 (21%)	4 (22%)	5 (24%)	6 (25%)

Hardy et al.[127]

	Males				Females			
Cyclohexylamine HCl:	**0**	**0.03%**	**0.1%**	**0.3%**	**0**	**0.03%**	**0.1%**	**0.3%**
N	46	45	31	46	44	46	42	44
Lymphosarcomas	1 (2%)	0 (0%)	1 (3%)	1 (2%)	0 (0%)	2 (4%)	3 (6%)	2 (4%)
Reticular cell neoplasms	0 (0%)	1 (2%)	1 (3%)	0 (0%)	4 (9%)	3 (7%)	3 (6%)	3 (7%)
Both	1 (2%)	1 (2%)	2 (6%)	1 (2%)	4 (9%)	5 (11%)	6 (13%)	5 (11%)

Kroes et al.[60]

	P generation males			F3b males			F6a males		
Sodium Cyclamate:	**0**	**2%**	**5%**	**0**	**2%**	**5%**	**0**	**2%**	**5%**
N	40	41	41	45	47	44	48	50	46
Lymphosarcomas	0 (0%)	1 (2%)	3 (7%)	0 (0%)	0 (0%)	2 (4%)	0 (0%)	6 (12%)	1 (2%)
Lymphosarcomas and Leukemias	2 (5%)	6 (15%)	8 (20%)	4 (9%)	2 (4%)	8 (18%)	5 (10%)	9 (18%)	2 (4%)

From Food and Drug Administration, Cyclamate (cyclamic acid, calcium cyclamate, and sodium cyclamate), Commissioner's decision, Fed. Reg. 45, 61474, September 16, 1980; Cancer Assessment Committee, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Scientific review of the long-term carcinogen bioassays performed on the artificial sweetener cyclamate, April 1984; Brantom, P. G., Gaunt, I. F., and Grasso, P., *Food Cosmet. Toxicol.*, 11, 735, 1973; Kroes, R., Peters, P. W. J., Berkvens, J. M., Verschuuren, H. G., DeVries, T., and Van Esch, G. J., *Toxicology*, 8, 285, 1977; Hardy, J., Gaunt, I. F., Hooson, J., Hendy, R. J., and Butterworth, K. R., *Food Cosmet. Toxicol.*, 14, 269, 1976. With permission.