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US EPA HPV Chemical Challenge Program

ROBUST SUMMARIES FOR TRICHLORO-S-TRIAZINETRIONE (CAS No. 87-90-1)

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ROBUST SUMMARIES FOR TRICHLORO-S-TRIAZINETRIONE
(CAS No. 87-90-1)

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Physical/Chemical Elements

1a

1 Melting Point / 2 Boiling Point

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: Purity: 99%

METHOD

• Method/Guideline Followed: OECD No. 102
• GLP: Not available
• Year: 1987

RESULTS

• Value in ^{o}C: 225-230 ^{o}C
• Decomposition: Yes
• Sublimation: No

CONCLUSIONS

Submitter's Comments: The endpoint has been adequately characterized in the EPA Reregistration Eligibility Document (RED) for Chlorinated Isocyanurates, September 1992.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (free text):

Chlorinated Isocyanurates Task 4, Registrant's Response to Product Chemistry Data Requirements, Submitted to EPA by Dynamac Corporation, August 4, 1992; and references therein.

Doshida, A., 1987, Trichloroisocyanuric Acid—Product Chemistry … Physical and Chemical Characteristics: Laboratory Project ID: 003/87. (Unpublished compilation prepared by Nippon Soda Co., Ltd. and received by EPA under 8033-4); MRID 40414403.

3a

Vapor Pressure

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: Purity: 99%

METHOD

• Method: Not available
• GLP: Not available
• Year: 1988

RESULTS

• Vapor Pressure Value: <1 mm Hg
• Temperature: 20 °C

CONCLUSIONS

Submitter's Comments: The endpoint has been adequately characterized in the EPA Reregistration Eligibility Document (RED) for Chlorinated Isocyanurates, September 1992.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (free text):

Chlorinated Isocyanurates Task 4, Registrant's Response to Product Chemistry Data Requirements, Submitted to EPA by Dynamac Corporation, August 4, 1992; and references therein.

Todhunter, J., 1988, Product Analysis Data and Certification of Limits for Cdf Chimie's Oniachlor 90, Oniachlor 60, Oniachlor EC TICA Granular and SDIC Granular: Project ID. DCF/PRODANAL.SUB, unpublished study prepared by Todhunter, Mandava, and Assoc. (Unpublished study submitted to EPA by Cdf Chime); MRID 40802401.

3b

Vapor Pressure

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method: Gas Saturation Procedure, 50 FR 39252 (Vol. 50, No. 188, printed September 27, 1985), equivalent to OECD 104 (Gas Saturation Procedure)
• GLP: Yes
• Year: 1991

RESULTS

• Vapor Pressure Value: Less than 0.002 Pa (less than 0.00005 mm Hg)
• Temperature: 20 °C

CONCLUSIONS

N/A

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (free text):

Trehy, M. L. and Adamove, J. E., Determination of the Vapor Pressure of ACL 90, Monsanto Co. Report MSL-11206, June 25, 1991.

Trehy, M. L. and Adamove, J. E., Determination of ACL 90 by LC with UV Detection, Monsanto Co. Report MSL-11197, June 25, 1991.

4a

Partition Coefficient

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method: OECD 107
• GLP: N/A
• Year: 1988

RESULTS

• Log Pow Value: Very small. Products are polar organics, which are insoluble in octanol and soluble in water. Not applicable. Products hydrolyze in water liberating chlorine and cyanuric acid.

CONCLUSIONS

Submitter's Comments: According to US EPA (Chlorinated Isocyanurates Task 4, Registrant's Response to Product Chemistry Data Requirements), "because the chlorinated isocyanurates are polar compounds, data pertaining to octanol/water partition coefficient are not required" and this endpoint is considered to be adequately characterized.

REFERENCES (Free Text):

Chlorinated Isocyanurates Task 4, Registrant's Response to Product Chemistry Data Requirements, Submitted to EPA by Dynamac Corporation, August 4, 1992; and references therein.

Mandava, N.; Todhunter, J., 1988, Hi-Lite 90P …: Product Chemistry: Physical and Chemical Characteristics and Other Requirements, unpublished study prepared by Nissan Chemical Industries Ltd.; MRID 40933106.

4b

Partition Coefficient

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- dissolves in water and dissociates to release available chlorine in the form of hypochlorous acid (HOCl). The HOCl is highly reactive and has a short half-life in contact with proteins and enzymes. Once all the available chlorine has reacted the only stable residual compounds are chloride ion and cyanuric acid.

METHOD

• Method: Similar to OECD 107
• GLP: No
• Year: 2000

RESULTS

• Value: The partition coefficient for s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- was determined to be 0.26. A partition coefficient of 0.067 is also reported for cyanuric acid.
• Temperature: Room temperature

CONCLUSIONS

Author's Comments: The octanol/water partition coefficient is 0.26 for s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-. Some available chlorine is lost by reaction with octanol during extraction.

DATA QUALITY

• Remarks field for Data Reliability: P_{OW} determined from measured concentrations in water only, because the concentration in octanol could not be measured.

REFERENCES (Free Text):

Kuechler, T. C., Occidental Chemical Corp. Memo titled "Octanol/Water Partition Coefficient for ACL® 90, ACL 60, and Isocyanuric Acid", May 15, 2000.

5a

Water Solubility

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method: OECD 105, OECD 112, ASTM-E70-74
• GLP: Not available
• Year: 1987

RESULTS

• Value:
1.2 g/100 g water, 25 °C
500 ppm hexane, 25 °C
2.5 g/100g benzene, 25 °C
25 g/100 g acetone, 25 °C
0.9 g/100 g chloroform, 25 °C
11.5 g/100 g dioxane, 25 °C
800 ppm in petroleum ether, 25 °C
500 ppm carbon tetrachloride, 25 °C
• pH Value and Concentration at Temperature: 2.75 (1% solution)
• pKa Value at 25 °C: 1.38×10^{-4}

CONCLUSIONS

Submitter's Comments: The endpoint has been adequately characterized in the EPA Reregistration Eligibility Document (RED) for Chlorinated Isocyanurates, September 1992.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (Free Text):

Doshida, A., 1987, Trichloroisocyanuric Acid - Product Chemistry … Physical and Chemical Characteristics: Laboratory Project ID: 003/87, (unpublished compilation prepared by Nippon Soda Co., Ltd. and received by EPA under Registration Number 8033-4); MRID 40414403.

REMARKS FIELD FOR GENERAL REMARKS:

CYANURIC ACID - Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products. The following data provides information on cyanuric acid and is provided here as support for s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1.

CYANURIC ACID RESULTS:

2 g/L water, 25 °C
2010 mg/L in water, 23 °C
7 g/L water, 50 °C
25 g/L water, 90 °C
0.1% in alcohol, 22 °C
7.2 g/100 g DMF, 25 °C
1959 mg/L in 8% ethanol, 23 °C
2440 mg/L in 50% ethanol, 23 °C
690 mg/L in 95% ethanol, 23 °C
410 mg/L in 100% ethanol, 23 °C
2330 mg/L in 3% acetic acid, 23 °C
3 mg/L in heptane, 23 °C
134 mg/L in wet octanol, 23 °C
14 mg/L in corn oil, 23 °C

REFERENCES:

Chlorinated Isocyanurates Task 4, Registrant's Response to Product Chemistry Data Requirements, Submitted to EPA by Dynamac Corporation, August 4, 1992; and references therein.

Isocyanurates Task 1: Product Chemistry Chapter; Submitted to EPA by Dynamac Corporation, June 8, 1987.

Nelson, G.D., 1967, The fundamental properties of cyanuric acid and the isocyanurates and their roles in maintaining pool water quality; MRID 19400.

ICI Americas, Inc., Chemical Analysis of TICA, SDIC-G, TICA-Tablet and ICA-G. Includes undated methods. (Unpublished study received by EPA Aug. 1, 1978 under 10182-10; CDL: 234648-A.); MRID 20465.

Kuechler, T. C., and Keller, U., Occidental Chemical Corp. Memo titled "Solubility Data", June 22, 1995.

Environmental Fate and Pathway Elements

6a

Photodegradation

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: Other (measured)
• Type: Water
• GLP: No
• Year: 1981
• Light Source: Continuous simulated sunlight irradiation
• Light Spectrum: Not stated
• Spectrum of Substance: Not stated
• Remarks field for Test Conditions:
- Test Medium: Water
- Controls: Distilled water
- Duration: 30 days
- Test Conditions: In order to determine the rate of photolytic degradation, phosphate buffer solutions (pH 7.5) containing 100 mg/l monosodium cyanurate were prepared using distilled water, laboratory tap water and non-chlorinated well water. Each was then exposed to a simulated sunlight source (consisting of a 20-watt GE fluorescent blacklight) for a continuous period of 30 days at a temperature of 25 ^{o}C. Analyses were carried out using pulse polarographic techniques.

RESULTS

• Concentration of substance: 100 mg/l
• Temperature: 25 ^{o}C
• Direct Photolysis:
- Halflife t1/2: >30 day
- Degradation: 0 % after 30 day
• Indirect Photolysis
- Sensitizer (type): N/A
- Concentration of Sensitizer: N/A
- Rate Constant: N/A
- Degradation % after: N/A
- Breakdown products: N/A
• Remarks field for Results: Pulse polarographic determinations made on the photolysis solutions up to and including 30 days of exposure showed no degradation of the test compound in any of the three water sources used. As a consequence, no photolytic half-life calculations were possible.

CONCLUSIONS

Author's Comments: A visual examination of these data indicate that there is no significant change in the concentration of 2,4,6-trihydroxy-1,3,5-triazine in both the sample and control solutions over the 30 day period. It is therefore concluded that no photodegradation occurred during the experimental period.

REFERENCES (Free text):

Hu, H.C.; "Photodegradation Study of Aqueous Sodium Salt Solutions of 2,4,6-Trihydroxy-1,3,5-Triazine; Unpublished Study Conducted by Center Analytical Services, Princeton Chemical Research & Development Center, Princeton, NJ 08540 for the Isocyanurate Industry Ad Hoc Committee (EPA Consortium 55643); Study No. (none), January 21, 1981. MRID 00056479.

7a

Stability in Water

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline Followed: Not Stated
• Type: Abiotic
• GLP: No
• Year: 1981
• Remarks field for Test Conditions: The abiotic hydrolysis of monosodium cyanurate was studied at three concentrations (50, 100 and 200 mg/l) in three pH buffers (pH 5, 7 and 9) and in three sources of water (distilled water, laboratory tap water and non-chlorinated well water). The tests were carried out in the dark at 25 ^{o}C for a period of 30 days. Test solutions were analyzed using a pulse polarographic technique.

RESULTS

• Nominal: N/A
• Measured Value: N/A
• Degradation % at a specified pH and temperature: None
• Half Life:
t1/2 at pH 7: >30 day at 25 ^{o}C
t1/2 at pH 9: >30 day at 25 ^{o}C
t1/2 at pH 5: >30 day at 25 ^{o}C
• Breakdown products: No

CONCLUSIONS

Author's comments: Pulse polarographic analysis of each of the test solutions showed that no chemical changes occurred during the 30-day test period. Monosodium cyanurate does not undergo hydrolysis in the dark at 25 ^{o}C at pH ranges from 5 through 9 over a period of 30 days.

REFERENCES (Free text):

Hu, H.C.; "Hydrolysis Study of Aqueous Sodium Salt Solutions of 2,4,6-Trihydroxy-1,3,5-Triazine; Unpublished Study Conducted by Center Analytical Services, Princeton Chemical Research & Development Center, Princeton, NJ 08540 for the Isocyanurate Industry Ad Hoc Committee (EPA Consortium 55643); Study No. (none), January 16, 1981. MRID 00056478.

8a

Transport and Distribution

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Test (test type): Adsorption/desorption
• Method (Y/N): Comparable to OECD Method 106
• Year (study performed): 1982
• Remarks field for Test Conditions: The soil partition coefficient of cyanuric acid was measured on various soils by equilibrating an aqueous solution of radio-labeled cyanuric acid with each soil for 24 hours. The concentrations in the water and soil were determined by scintillation counter.

RESULTS

• Media: Water, soil (4 types)
• Remarks field for Results: The average organic carbon partition coefficient was: Koc (average of 4 soils) = $[H_3Cy]$soil x 100% / $[H_3Cy]$water x % org. C = 51.

CONCLUSIONS

Author's Comments: The cyanuric acid soil/water partition coefficient indicated that cyanuric acid is weakly adsorbed and highly mobile in soils and sediments.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (Free Text)

Michael, P., and Cummings, I., "Cyanuric Acid Absorption On Soils", Monsanto Report ES-82-SS-94, December, 1982.

8b

Transport and Distribution

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Test (test type): N/A
• Method (Y/N): Other
• Year (study performed): 2001
• Remarks field for Test Conditions:
- Fugacity calculated using EPIWIN (Estimation Program Interface for Windows) 3.05; US EPA version for Windows.
- Input parameters were as follows:
Melting point: >330 ^{o}C
Boiling point: >300 ^{o}C
Aqueous solubility: 2010 mg/L at 25 ^{o}C
Kow range: 0.067 to 0.26
log Kow range: -1.174 to -0.585

RESULTS

• Media: air, water, soil and sediment
• Estimated Distribution and Media Concentration (levels II/III):
Air: <0.1%
Water: 99.8%
Soil: <0.1%
Sediment: 0.17%
• Remarks field for Results:
- Data calculated by EPIWIN:
Vapor Pressure: 1.8 E-6 Pascals
log Kow: -1.17 (provided value)
Aqueous Solubility: 2010 mg/L (provided value)
Henry's constant: 1.14 E-12 atm-m3/mol (calculated from above vapor pressure and solubility)
Hydroxyl radical-mediated second order rate constant: 0.47 E-12 cm3/molecule-sec
Hydroxyl radical-mediated t1/2: 22.8 days (assuming 12 hour day, 1.5 E+6 HO molecules per cm3)
Soil Koc: 124 L/kg
Hydrolysis cannot be estimated for this structure.
BCF = 3.2 L/kg
Level 3 distribution modeling: t1/2 air: 547 hr; t1/2 water and soil: 360 hr; t1/2 sediment: 1440 hr.
Assumed that 100% went to water.

CONCLUSIONS: N/A

DATA QUALITY

• Remarks field for Data Reliability: Estimated value based on accepted model

REFERENCES (Free Text)

Fugacity Model (Cyanuric Acid)

9a

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both compounds.

METHOD

• Method: Literature review
• Type: Aerobic and anaerobic
• GLP: Not applicable
• Remarks field for Test Conditions: See results

RESULTS

• Degradation: See conclusions
• Results: Aerobic: degrades very slowly except for specific fungi or bacteria which have been acclimated to cyanuric acid; anaerobic: inherently biodegradable
• Breakdown products: Aerobic: carbon dioxide and ammonia; anaerobic: carbon dioxide and ammonia

CONCLUSIONS

Submitter's Comments: This report is a comprehensive literature review, with 22 references, of the biodegradation of cyanuric acid under a variety of conditions.

Aerobic biotransformation -
Cyanuric acid normally degrades very slowly under aerobic conditions. However, it can be degraded if specific fungi or bacteria strains are present, the microorganisms have been acclimated to cyanuric acid, and organic nutrients are present.

Only one (the fungus Sporothrix schenckii) of 160 microorganisms tested by Zeyer gave significant degradation of cyanuric acid under aerobic conditions. They found rapid degradation to carbon dioxide and ammonia and that all of the liberated ammonia was incorporated into biomass. The soil fungi Stachybotrys chartarum, Hendersonula toruloidea, Penicillium sp. and Hormodendrum masonii have also been reported to degrade cyanuric acid.

Several types of bacteria, including an unidentified mixed culture, Pseudomonas sp., and Achromobacter sp. can degrade cyanuric acid. The bacteria grew under aerobic conditions with cyanuric acid as the only source of nitrogen. Cyanuric acid is readily degraded in the aerobic, activated sludge process used by the municipal wastewater treatment plant receiving waste from a chlorinated isocyanurate manufacturing plant in Illinois, USA. The extent of degradation averages about 85%. Weber and coworkers report that cyanuric acid is readily degraded in aerated, activated sludge under nitrogen-limiting conditions.
Cyanuric acid is not an energy source for the bacteria, since the degradation is a hydrolysis. Cyanuric acid is converted into carbon dioxide and ammonia. The ammonia is then incorporated into the biomass or nitrified.

A solution of cyanuric acid has a BOD_5 of 0.0065 mg/L per mg/L of cyanuric acid and a COD of 0.011 mg/L per mg/L of cyanuric acid.

Anaerobic biotransformation -
Saldick found that cyanuric acid biodegrades readily under a wide variety of natural conditions, and particularly well in systems of either low or zero dissolved oxygen level, such as anaerobic activated sludge and sewage, soils, mud, and muddy streams and river water, as well as ordinary aerated activated sludge systems with typically low (1 to 3 ppm) dissolved oxygen levels.

Cyanuric acid is biodegraded under anaerobic conditions by some strains of bacteria, such as Pseudomonas, Klebsiella pneumoniae, and others, into carbon dioxide and ammonia. Eaton and Karns located the gene that encodes the cyanuric acid aminohydrolase enzyme responsible for hydrolysis of cyanuric acid in three strains of bacteria.

Cyanuric acid is also taken up and degraded by plants. Cyanuric acid acts as a slow release nitrogen fertilizer, although it showed some toxicity to plants at higher levels, similar to some other nitrogen fertilizers.

DATA QUALITY

• Remarks field for Data Reliability: This paper reviews a significant number of published papers on biodegradation.

REFERENCES (Free text):

Occidental Chemical Corporation, March 22, 2001, Biotransformation of Isocyanuric Acid; and the following references therein.
An Index of Refractory Organics, T. Helfgott, F. Hart and R. Bedard, EPA 600/2-77-174, 1977, pp. 1-7, 24-25.
Process for the biological degradation of cyanuric acid, J. Zeyer, R. Hütter, and P. Mayer, UK Patent App. 2,025,919 (Jan. 30, 1980).
Rapid Degradation of Cyanuric Acid by Sporothrix schenckii, J. Zeyer, J. Bodmer and R. Hütter, Zentrabl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Orig. C, 1981, 2(2) 99-110.
Microbial Decomposition of Ring-14C Atrazine, Cyanuric Acid, and 2-Chloro-4,6-diamino-s-triazine, D. Wolf and J. Martin, J. Environ. Qual., 1975, 4(1) 134-139.
Cyanuric Acid as Nitrogen Source for Micro-Organisms, H. Jensen and A. Abdel-Ghaffar, Arch. Mikrobiol., 1969, 67, 1-5.
Biological Treatment Specific for an Industrial Wastewater Containing s-Triazines, W. Hogrefe, H. Grossenbacher, A. Cook and R. Hütter, Biotech. and BioEng., 1985, 27, 1291-1296.
Degradation of Cyanuric Acid by Immobilized Bacteria, C. Ernst and H. Rehm, DECHEMA Biotechnol. Conf., 4(Pt. A), 1990, 577-580.
s-Triazines as Nitrogen Sources for Bacteria, A. Cook and R. Hütter, J. Agric. Food Chem., 1981, 29(6) 1135-1143.
Oxidation of simazine: Biological oxidation of simazine and its chemical oxidation products, M. Lai, A. Weber, and J. Jensen, Water Env. Res., 1995, 67(3) 347-354.
Continuous Culture Biodegradation of Simazine's Chemical Oxidation Products, S. Sisodia, A. Weber, and J. Jensen, Water Research, 1996, 30(9) 2055-2064.
Biodegradation of Cyanuric Acid, J. Saldick, Applied Microbiology, 1974, 28(6) 1004-1008.
Ring cleavage and degradative pathway of cyanuric acid in bacteria, A. Cook, P. Beilstein, J. Grossenbacher, and R. Hütter, Biochem. J., 1985, 231, 25-30.
Anaerobic Degradation of Cyanuric Acid, Cysteine, and Atrazine by a Facultative Anaerobic Bacterium, J. Jessee, R. Benoit, A. Hendricks, G. Allen, and J. Neal, Appl. and Environ. Microbiol., 1983, 45(1) 97-102.
Cloning and Comparison of the DNA Encoding Ammelide Aminohydrolase and Cyanuric Acid Amidohydrolase from Three s-Triazine-Degrading Bacterial Strains, R. Eaton and J. Karns, J. Bacteriol., 1991, 173(3) 1363-1355.
Fate of 14C-labeled triazine herbicides in plants, P. Müller and P. Payot, Proc. IAEA Symp. Isotopes and Weed Res., IAEA, Vienna, Austria, 1966, 61-70.
Crop Response to Urea and Urea Pyrolysis Products, G. Terman, et al., J. Agric. Food Chem., 1964, 12(2) 151-154.
Occidental Chemical Corporation, unpublished data.

9b

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method: The method employed for measuring growth is described in J. Biol. Chem. 195, 265-275 (1951)
• Type: Aerobic
• GLP: Not specified
• Contact time: Up to 54 h
• Innoculum: Pseudomonas sp. (NRRL B-12228) and Achromobacter sp. (bacterium isolated from a maize field)
• Remarks field for Test Conditions:
- Concentration of test chemical: 250-2000 mg/l
- Temperature: 25-35°C
- Sampling frequency: Variable
- Controls and blanks: Non-biological decomposition was not detected in a control
- Analytical methods: Cyanuric acid was determined by HPLC (Lichrosorb RP 8 column, 1 ml/min of a 10 mM potassium phosphate buffer at pH 6.7 mobile phase) with UV detection at 220 nm
-Method of calculating measured concentrations: All data reported

RESULTS

• Degradation:
Ca alginate entrapped Pseudomonas sp.: 30% in 48 h
Ca alginate entrapped Achromobacter sp.: 15% in 48 h
Free cells of Pseudomonas sp.: specific degradation rate = 70-360 mg/h/g
Free cells of Achromobacter sp.: specific degradation rate = 75-275 mg/h/g
• Results: free cells showed higher degradation rates than immobilized bacteria
• Kinetic: Degradation rates for immobilized cells were independent of cyanuric acid concentration
• Breakdown products: Carbon dioxide and ammonia

CONCLUSIONS

Author's Comments: In Ca alginate entrapped cells of Pseudomonas sp. NRRL B-12228 and Achromobacter sp. are able to grow with cyanuric acid as sole nitrogen source and to degrade this compound completely to carbon dioxide and ammonia. Within aqueous solubility of cyanuric acid there could not be determined any toxicity limit for investigated bacteria. Comparing specific degradation rates of free and Ca alginate entrapped cells, immobilized microorganisms showed distinctly less decomposition qualities. Optimal temperature for the utilization of cyanuric acid is 35 °C for both bacteria. In cultures of immobilized cells of Pseudomonas sp. B-12228 the greatest amount of cyanuric acid was degraded during logarithmic phase. After 54 h no considerable differences resulted in decomposed cyanuric acid. In cultures of Achromobacter sp. the course of degradation was almost linear, in all events there was the same amount of the triazine utilized after 48 h.

DATA QUALITY

• Remarks field for Data Reliability: This study provides useful information on the bacterial degradation of cyanuric acid and in combination with the other published studies adequately addresses this endpoint.

REFERENCES (Free text):

Degradation of Cyanuric Acid by Immobilized Bacteria, C. Ernst and H. Rehm, DECHEMA Biotechnol. Conf., 4(Pt. A), 1990, 577-580.

9c

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No.: 108-80-5
• Remarks field for Test Substance:
- Purity: 98%
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method: Multiple tubes of medium (either cyanuric acid and cysteine (CA-CTS) or cyanuric acid and FeS (CA-FeS)) were inoculated. Six tubes were selected at each sampling time. Duplicate samples were obtained by pooling three tubes each. Enrichment cultures were initiated by placing 1 g of sediment in 10 ml of CA-CYS medium into anaerobic culture tubes. The cultures were incubated and transferred every two weeks before isolation of pure cultures were attempted.
• Type: Anaerobic and aerobic
• GLP: N/A
• Contact time: 7-8 days
• Inoculum: Pure culture of a facultative anaerobic bacteria (gram-negative) isolated from stream sediment acclimated to cyanuric acid
Remarks field for Test Conditions:
- Concentration of test chemical: 1500 mg/l
- Temperature: 30 °C
- Sampling frequency: 0.5-1 per day
- Controls and blanks: No degradation in uninoculated controls
- Analytical method: Cyanuric acid was determined in filtered samples by HPLC, ammonia was determined by the phenate method
- Method of calculating measured concentrations: Arithmetic mean of duplicate samples

RESULTS

• Degradation:
The faculatively anaerobic bacterium degraded cyanuric acid under anaerobic conditions.
in cysteine media: 100% in 4 d
in FeS media: 57% in 8 d
• Breakdown products: Ammonia

CONCLUSIONS

Author's Comments: A facultative anaerobic bacterium that rapidly biodegrades cyanuric acid was isolated from the sediment of a stream that received industrial wastewater effluent. Cyanuric acid decomposition was measured throughout the growth cycle by using an HPLC assay and the concomitant production of ammonia was also measured. The facultative anaerobic bacterium isolated in this study degraded cyanuric acid under anaerobic, but not aerobic, conditions. Other cyanuric acid degrading bacteria, including a sulfate-reducing bacteria, were also isolated but not studied in detail.

DATA QUALITY

• Remarks field for Data Reliability: The study relies on sediment taken from a stream that receives industrial wastewater and while no attempt was made to measure the species diversity of the bacteria, the study reliably shows that the potential to degrade cyanuric acid is widespread among a broad spectrum of bacterial species.

REFERENCES (Free text):

Anaerobic Degradation of Cyanuric Acid, Cysteine, and Atrazine by a Facultative Anaerobic Bacterium, J. Jessee, R. Benoit, A. Hendricks, G. Allen, and J. Neal, Appl. and Environ. Microbiol., 1983, 45(1) 97-102.

9d

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method: J. Water Pollution Control Fed., 39, 181-187 (1967); J. Water Pollution Control Fed., 40, 306-319 (1968)
• Type: anaerobic
• GLP: Not specified
• Contact time: N/A
• Inoculum: three strains of bacteria: Pseudomonas NRRL B-12227 and B-12228 and Klebsiella pneumoniae strain 99
• Remarks field for Test Conditions:
- Concentration of test chemical: 0.83 mM (108 mg/l)
- Other: Cyanuric acid, biuret, urea, or ammonium was the sole source of combined nitrogen
- Controls and blanks: Cyanuric acid was stable in sterile growth medium, organisms did not grow in the absence of a source of combined nitrogen
- Analytical method: Cyanuric acid and biuret were determined by reverse phase HPLC, CO_2 was determined manometrically after acidification of the reaction mixture, ammonium was measured by the Berthelot reaction

RESULTS

• Degradation: 100%, with all s-triazine nitrogen incorporated into cell material
• Kinetic: Specific degradation rate for bacteria = 0.5-1.3 mkat/kg of protein;
specific degradation rate for crude enzyme extracts = 2.2-10 mkat/kg of protein
• Breakdown products: Carbon dioxide and ammonia (via biuret and urea intermediates)

CONCLUSIONS

Author's Comments: The degradative pathway of cyanuric acid was examined in Pseudomonas sp. strain D. The bacteria grew with cyanuric acid, biuret, urea, or ammonium as sole source of nitrogen, and each substrate was entirely metabolized concomitantly with growth. Cyanuric acid was converted stoichiometrically into ammonium ion and CO_2 via biuret and urea intermediates. The reactions proceeded under aerobic or anoxic conditions and were presumed to be hydrolytic. Data indicated that the same pathway occurred in another pseudomonad and a strain of Klebsiella pneumoniae.

Crude extracts of the active degradation enzymes from each bacteria strain were isolated and tested.

DATA QUALITY

• Remarks field for Data Reliability: Study appears well conducted and was published in a respected peer-reviewed journal. The information combined with the other biodegradation studies adequately addresses this endpoint.

REFERENCES (Free text):

Ring cleavage and degradative pathway of cyanuric acid in bacteria, A. Cook, P. Beilstein, J. Grossenbacher, and R. Hütter, Biochem. J., 1985, 231, 25-30.

9e

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both compounds.

METHOD

• Method: Study was conducted in accordance with published test methods emp loying highly aerated systems and is described in "A biodegradability test for organic compounds. (1967) J. Water Pollut. Contr. Fed. 39:181-187; Biodegradability of organic chemicals isolated from rivers. (1963) Proc. 18th Ind. Waste Conf. Purdue Univ. 115:278-282.
• Type: Anaerobic and aerated activated sludge
• GLP: Not specified
• Contact time: Ranged from hours to many days
• Inoculum: Activated sludge maintained in the laboratory in continuous-flow units using either raw or pasteurized domestic primary settled sewage as feed
• Remarks field for Test Conditions:
- Concentration of test chemical: 0.4-40 mg/l
- Controls and blanks: Experiments without mud or sewage organisms gave no detectable $^{14}CO_2$ yield.
- Analytical method: Gravimetric analysis for cyanuric acid by precipitation with melamine or ^{14}C-labeled radio-tracer analysis for evolved carbon dioxide

RESULTS

• Degradation:
By aerated activated sludge: 100% after 8 h (2.5 mg/l dissolved O_2); 42% after 9 h (8.7 mg/l dissolved O_2)
In highly aerobic systems: 0% degradation
In anaerobic sewage: 25-50% in 48 h, 100% in 72 h
In anaerobic mixed liquor: Variable results, 70-90% in 6 h to 13 d
In anaerobic nutrient broth: 95-98% in 72 h
In 6 types of soils and muds: 52-100% in 9-23 d
• Breakdown products: Carbon dioxide and ammonia
• Remarks field for Results:
- In aerated activated sludge: More consistent degradation results were obtained when nutrients were added.
- In anaerobic sewage: The amount of ammonia and Kjeldahl nitrogen produced is accounted for by the amount of cyanuric acid degraded, within analytical error.

CONCLUSIONS

Author's Comments: Cyanuric acid biodegrades readily under a wide variety of natural conditions, and particularly well in systems of either low or zero dissolved oxygen level, such as anaerobic activated sludge and sewage, soils, muds, and muddy streams and river waters, as well as ordinary aerated activated sludge systems with typically low (1 to 3 ppm) dissolved oxygen levels. The overall degradation reaction is merely a hydrolysis; carbon dioxide and ammonia are the initial hydrolytic breakdown products. Since no net oxidation occurs during this breakdown, biodegradation of cyanuric acid exerts no primary biological oxygen demand. However, eventual nitrification of the ammonia released will exert its usual biological oxygen demand. Organisms which degrade cyanuric acid under anaerobic conditions do not require any acclimatization to be active. In highly aerobic media, cyanuric acid resists biodegradation.

DATA QUALITY

• Remarks field for Data Reliability: Study appears well conducted and was published in a respected peer-reviewed journal. The information combined with the other biodegradation studies adequately addresses this endpoint.

REFERENCES (Free text):

Biodegradation of Cyanuric Acid, J. Saldick, Applied Microbiology, 1974, 28(6) 1004-1008.

9f

Biodegradation

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both compounds..

METHOD

• Method: s-triazine biodegradation was investigated as a function of continuous culture process growth rate.
• Type: Continuous culture under aerobic conditions
• GLP: Not specified. The study was published in a respected peer-reviewed journal.
• Year: 1996
• Contact time (units): 1-7 days
• Inoculum: Mixed culture from batch reactor acclimated to s-triazines as the nitrogen source
• Remarks field for Test Conditions:
- Concentration of test chemical: Continuously added at a influent concentration of 0.92 mg/l in a mixture with other s-triazines
- Pre-acclimation conditions: Chemostats operated for 1-4 months at the desired contact time before data collection
- Temperature: 25 °C (± 2)
- Dosing procedure: Chemostat feed was prepared daily, influent flow rates were calibrated daily
- Sampling frequency: Samples taken on seven consecutive days and analyzed in duplicate
- Controls and blanks: Removal by volatilization and sorption were assessed and found to be minimal
- Analytical method: Filtered samples analyzed with HPLC using a C18 column and UV detection at 210 nm, mobile phase was 0.01 M phosphate buffer at pH 7.2
- Method of calculating measured concentrations: Arithmetic mean of 14 measurements

RESULTS

• Degradation: 100% at net growth rates ranging from 1.0 to 0.14 d^{-1}
• Breakdown products: Carbon dioxide and ammonia (ammonia was assimilated into biomass)

CONCLUSIONS

Author's Comments: Cyanuric acid and other s-triazines are metabolized as nitrogen sources for microbial growth under nitrogen limiting conditions. The presence of other nitrogen sources (including ammonium and nitrate) negatively influence the rate of cyanuric acid degradation. Cyanuric acid is competitive with ammonium and nitrate, indicating that degradation of cyanuric acid might be accomplished under conditions where nitrogen is not limiting. Percent degradation was inhibited at low nutrient concentrations.

DATA QUALITY

• Remarks field for Data Reliability: Study appears well conducted and was published in a respected peer-reviewed journal. The information combined with the other biodegradation studies adequately addresses this endpoint.

REFERENCES (Free text):

Continuous Culture Biodegradation of Simazine's Chemical Oxidation Products, S. Sisodia, A. Weber, and J. Jensen, Water Research, 1996, 30(9) 2055-2064.

Ecotoxicity Elements

10a

Acute Toxicity to Fish

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: white granules, stored at room temperature in the dark. Sample purity was specified as greater than 99%.

METHOD

• Method/Guideline followed: US EPA-TSCA, 40 CFR Part 797.1400
• Type (Test Type): Static bioassay
• GLP (Y/N): Yes
• Year Study Performed: 1987
• Species/Strain/Supplier: Bluegill sunfish (Lepomis macrochirus) from Osage Catfisheries, Inc., Osage Beach, Missouri
• Analytical Monitoring: N/A
• Exposure Period (unit): 24 h, 48 h, 96 h
• Statistical Methods: (1) A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript. August 1978. Stephan, CE, KA Busch, R Smith, J Burke and RW Andrew.
(2) Methods for calculating an LC50, pp. 65-84. In FL Mayer and JL Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.
• Remarks field for Test Conditions:
- Procedures Used: (1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA, Ecological Research Series EPA-660/3-75-009, April 1985, 61 p. (2) Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 1990. 15th ed. Washington, DC. 1134 p.
- Test fish mean weight: 0.50 (+/- 0.14) g; mean length: 26.0 (+/- 2.3) mm; test chamber loading biomass: 0.33 g/L; pretreatment: all test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. Fish culture techniques used were basically those described by Brauhn et al (Brauhn JL and RA Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in the Raw Data. During the period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (Artemia sp.) daily until 48-96 hours prior to testing at which time feeding was discontinued.
- Details of Test: Static
- Dilution Water Source: Well water
- Dilution Water Chemistry: Hardness: 50-56 mg/l as $CaCO_3$
- Alkalinity: 34-38 mg/l as $CaCO_3$
- Initial pH: 7.3-7.6
- DOC: 9.7 mg/L at 0-hour
- pH at 0-hour: 7.6
- Fish per concentration: 10
- Stock and Test Solution and how they are prepared: The definitive test concentrations were obtained by transferring appropriate aliquots of a working standard directly to the test chambers. The working standard was prepared in 50 ml of dimethylformamide (DMF) for a concentration of 18 mg test compound per milliliter of solvent. All test concentrations were based on the total compound, *i.e.*, not corrected for sample purity. The solvent control chamber received a 1.5 ml aliquot of dimethylformamide, which was equivalent to the highest amount used in any test solution.
- Exposure vessel: five gallon glass vessel containing 15 L soft reconstituted water kept in water bath; no aeration during testing
- Water Chemistry in test: DOC and pH are consistent for control and concentrations where effects are seen. Control DOC at 96h = 6.1 mg/L; control pH at 96h = 6.9. 96h DOC for 0.32 mg/l = 6.0 mg/L; 96h pH for 0.32 mg/L = 6.9.
- Test Temperature Range: 22 ^{o}C (+/-1.0)

RESULTS

• Nominal concentrations: 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L
• Measured Concentrations: No Analysis Performed
• Unit (results expressed in what unit): mg/L
• Element value: LC50 @ 24h, 48h and 96h: 0.40 mg/L; 96h NOEC estimated to be 0.18 mg/L
• Statistical Results: 95% confidence limits (0.32-0.56 mg/L)
• Remarks field for Results:
- Table Showing Cumulative mortality:

Nominal Concentration (mg/L)	24h Percent Mortality	48h Percent Mortality	96h Percent Mortality
Control	0	0	0
Solvent Control	0	0	0
0.18	0	0	0
0.32	10	10	10
0.56	100	100	100
1.0	100	100	100
1.8	100	100	100

- Lowest test substance concentration causing 100% mortality: 24h, 48h and 96h: 0.56 mg/L
- Mortality of controls: 0%
- Abnormal Responses: Mortality observed in the 0.32, 0.56, 1.0 and 1.8 mg/L concentrations during 96h exposure period.

CONCLUSION

Author's Comments: The results indicated a 96-hour, no-observed effect concentration could be estimated at 0.18 mg/l, which was based on the lack of mortality and abnormal effects at the lowest concentration tested.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (Free text):

Bowman, J. H., Acute Toxicity of ACL 90+ to Bluegill Sunfish (Lepomis macrochirus), Study Conducted by Analytical Biochemistry Laboratories, Inc., Columbia, MO 65202 for Monsanto Company, Project No. 35462, February 15, 1987.

10b

Acute Toxicity to Fish

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks: White granules, stored at room temperature in the dark. Sample purity was specified as 99%.

METHOD

• Method/Guideline followed: US EPA-TSCA, 40 CFR Part 797.1400
• Type (Test Type): Static bioassay
• GLP (Y/N): Yes
• Year Study Performed: 1987
• Species/Strain/Supplier: Rainbow trout from Spring Creek Trout Hatchery, Lewistown, Montana, USA
• Analytical Monitoring: N/A
• Exposure Period (unit): 24 h, 48 h, 96 h
• Statistical Methods: (1) A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript. August 1978. Stephan, CE, KA Busch, R Smith, J Burke and RW Andrew. (2) Methods for calculating an LC50, pp. 65-84. In FL Mayer and JL Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. ASTM Special Technical Publication 634. ASTM. Philadelphia.
• Remarks field for Test Conditions:
- Procedures Used: (1) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA, Ecological Research Series EPA-660/3-75-009, April 1985, 61 p. (2) Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 1990. 15th ed. Washington, DC. 1134 p.
- Test fish: Mean weight: 0.76 (+/- 0.23) g; Mean length: 39.0 (+/- 4.0) mm; Test chamber loading biomass: 0.51 g/L; Pretreatment: all test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. Fish culture techniques used were basically those described by Brauhn et al (Brauhn JL and RA Schoettger. 1975. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May, 1975. 45 p.). A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in the Raw Data. During the period, the fish received a standard commercial fish food occasionally supplemented with brine shrimp nauplii (Artemia sp.) daily until 48-96 hours prior to testing at which time feeding was discontinued.
- Details of Test: Static
- Dilution Water Source: Well water
- Dilution Water Chemistry: Hardness: 40-48 mg/L as $CaCO_3$
- Alkalinity: 20-30 mg/L as $CaCO_3$
- Initial pH: 7.2-7.6
- DOC: 9.8 mg/L @ 0-hour
- Fish per concentration: 10
- Stock and Test Solution: The definitive test concentrations were obtained by transferring appropriate aliquots of a working standard directly to the test chambers. The working standard was prepared in dimethylformamide (DMF). All test concentrations were based on the total compound, *i.e.*, not corrected for sample purity. The solvent control chamber received a 1.5 mL aliquot of DMF, which was equivalent to the highest amount used in any test solution.
- Exposure vessel: Five gallon glass vessel containing 15 L of soft reconstituted water kept in water bath; no aeration during testing
- Water Chemistry in test: DOC and pH are consistent for control and concentrations where effects are seen. Control DOC at 0-hour = 9.8 mg/L; control pH = 7.5; 0.32 mg/L DOC at 0-hour = 9.7 mg/L; pH = 7.4
- Test Temperature Range: 12 ^{o}C (+/- 1.0)

RESULTS

• Nominal concentrations: 0.056, 0.10, 0.18, 0.32, 0.56 and 1.0 mg/L
• Measured Concentrations: No Analysis Performed
• Unit (results expressed in what unit): mg/L

• Element value: LC50 at 24h: 0.26 mg/L; LC50 at 48h: 0.24 mg/L; LC50 at 96h: 0.24 mg/L; 96 h NOEC estimated to be 0.056 mg/L
• Statistical Results: 95% confidence limits (0.18-0.32 mg/L)
• Remarks field for Results:

- Table Showing Cumulative mortality:

Nominal Concentration (mg/L)	24h Percent Mortality	48h Percent Mortality	96h Percent Mortality
Control	0	0	0
Solvent Control	0	0	0
0.056	0	0	0
0.10	0	0	0
0.18	0	0	0
0.32	90	100	100
0.56	100	100	100
1.0	100	100	100

- Lowest test substance concentration causing 100% mortality: 24h: 0.56 mg/L; 48h and 96h: 0.32 mg/L
- Mortality of controls: 0%
- Abnormal Responses: The abnormal effects of mortality, loss of equilibrium and/or fish on the bottom of test chamber were observed in the 0.10, 0.32, 0.56 and 1.0 mg/L test concentrations during the 96h exposure period

CONCLUSION

Author's/Submitter's Comments: The results indicated a 96-hour, no-observed effect concentration could be estimated at 0.056 mg/L, which was based on the lack or mortality and abnormal effects.

DATA QUALITY

• Reliabilities: Klimisch Code 1a (Reliable without restriction; US EPA-TSCA, 40 CFR Part 797.1400)

REFERENCES (Free text):

Bowman, J. H., Acute Toxicity of ACL 90+ to Rainbow Trout (Salmo gairdneri), Study Conducted by Analytical Biochemistry Laboratories, Inc., Columbia, MO 65202 for Monsanto Company, Project No. 35514, February 24, 1987.

10c

Acute Toxicity to Fish

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: The procedures used in this test were described in the American Public Health Association's "Standard Methods for the Examination of Water and Wastewater" (1975) and the U.S. EPA's "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians" (1975). Comparable to OECD Guideline 203 "Fish, Acute Toxicity Test".
• Type (Test Type): Static Bioassay
• GLP (Y/N): No
• Year Study Performed: 1978
• Species/Strain/Supplier: Bluegill Sunfish/Lepomis macrochirus/Osage Catfisheries, Inc.
• Analytical Monitoring: No analytical monitoring was conducted during the test.
• Exposure Period (unit): 96 hrs (4 days)
• Statistical Methods: LC_{50} calculated according to the method of Litchfield and Wilcoxon, 1949.
• Remarks field for Test Conditions:
- Test fish: mean weight 0.51 g and standard length 27.8 mm
- Test Conditions: All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least fourteen days prior to testing. The test vessels were kept in a water bath at 22 ^{o}C (±1).
- Details of Test: Static
- Dilution Water Source: Laboratory well water
- Dilution Water Chemistry: Dissolved oxygen: 8.4 mg/l, pH 7.8, total hardness 220 mg/l $CaCO_3$, and total alkalinity 210 mg/l $CaCO_3$
- Fish per concentration: 10 fish per concentration
- Stock and Test Solution: Before the stock solution was prepared, the standard was allowed to warm to room temperature (22 ^{o}C). The working solution was prepared in deionized water.
- Concentrations: 0 and 1,000 mg/l, with 10 fish per test concentration. (A preliminary range finding test indicated that the toxicity of cyanuric acid exceeded 1,000 mg/l.)
- Vehicle/Solvent and Concentrations: None
- Stability of the Test Chemical Solutions: Not reported
- Exposure vessel: five gallon glass vessels containing 15 liters of laboratory well water
- Replicates, fish per replicate: one replicate, 10 fish per replicate (The test fish were acclimated and held without food 48 hours prior to testing.)
- Water Chemistry in test: Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.
- Test Temperature Range: The test vessels were kept in a water bath at 22 ^{o}C (±1).

RESULTS

• Nominal concentrations: 0 and 1,000 mg/l
• Measured Concentrations: Not reported
• Endpoint value: >1,000 (measured value) = LC_{50} at 24, 48, and 96 hours

	LC_{50} in milligrams/l (ppm)		
Compound	24 hours	48 hours	96 hours
Cyanuric Acid	>1000	>1000	>1000
Antimycin A	0.00012 (0.000096-0.00015)	0.00096 (0.000079-0.00012)	0.000844 (0.000067-0.00010)

• Statistical Results: see chart showing LC_{50} values
• Remarks field for Results:
- Cumulative mortality:

Concentration (mg/l)	Observed Mortality %		
	24 hours	48 hours	96 hours
Control	0	0	0
1,000	0	0	0

- Lowest test substance concentration causing 100% mortality: no test concentration caused 100% mortality
- Mortality of controls: none observed
- Reference Substances: Antimycin A
- Any observations (i.e. precipitates): The dissolved oxygen concentration, which stayed between 40% and 100% saturation, was considered adequate for testing by the study directors. The pH values for all concentrations remained consistent with the controls throughout the test and ammonia concentrations were below the toxic limit. The observed 96 hour LC_{50} and 95% confidence limits for Antimycin A were within 95% confidence limits reported in the literature, which indicated to the study directors that the fish were in good condition.

CONCLUSION

Author's Comments: The study demonstrated that the predicted LC_{50} values for Cyanuric Acid is >1,000 mg/l.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 203.

REFERENCES (Free text):

Forbis, A.D. and Thompson, C.M.; Acute Toxicity of Cyanuric Acid (AB-78-1384330-2b) to Bluegill Sunfish (Lepomis macrochirus); Study Conducted by Analytical Biochemistry Laboratories, Inc., Columbia, MO 65205 for Monsanto Chemical Company; Project No. BN-78-377; September 12, 1978.

American Public Health Association, "Standard Methods for the Examination of Water and Wastewater", 14th edition, New York, 1975.

U.S. EPA, Committee on Methods for Toxicity Test with Aquatic Organisms: Ecol. Res. Ser. (C.E. Stephen Chairman), "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians", 660/3-75009, 1975.

10d

Acute Toxicity to Fish

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: The procedures used in this test were described in the American Public Health Association's "Standard Methods for the Examination of Water and Wastewater" (1975) and the U.S. EPA's "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians" (1975). Comparable to OECD Guideline 203 “Fish, Acute Toxicity Test”.
• Test Type: Static Bioassay
• GLP (Y/N): No
• Year Study Performed: 1978
• Species/Strain/Supplier: Fathead Minnows/Pimephales Promelas/Fattig Fish Hatchery(Brady, Nebraska)
• Analytical Monitoring: No analytical monitoring was conducted during this test.
• Exposure Period (unit): 96 hrs (4 days)
• Statistical Methods: Not stated in Report
• Remarks field for Test Conditions:
- Test fish: mean weight of 0.179 g and standard length of 20.9 mm
- Details of test: static
- Dilution water source: ABC Laboratory well water
- Dilution Water Chemistry: pH 7.8, total hardness 220 mg/l $CaCO_3$, and total alkalinity 210 mg/l $CaCO_3$
- Fish per concentration: 10 (The test fish were acclimated and held without food 48 hours prior to testing.)
- Stock and Test Solution and How Prepared: Before the stock solution was prepared, the standard was allowed to warm to room temperature (22 ^oC). The test concentrations were prepared by weighing appropriate amounts of Cyanuric Acid and transferring directly to the test chambers.
- Concentrations: 210, 370, 650, 1200, 2,100 mg/l
- Vehicle/Solvent and Concentrations: None
- Stability of the Test Chemical Solutions: not specified
- Exposure vessel: five gallon glass vessels containing 15 liters of laboratory well water.
- Number of replicates, fish per replicate: No repeat tests
- Water Chemistry in test: Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

- Test Temperature Range: The test vessels were kept in a water bath at 22 ^oC (±1).

RESULTS

• Nominal concentrations: 210, 370, 650, 1200, 2100 mg/l
• Measured Concentrations: not specified
• Unit: mg/l (ppm)
• Remarks field for Results:
- Acute lethal value: >2,100 mg/l = LC_{50} at 24, 48, and 96 hours
The following table presents the predicted LC_{50} values and 95% confidence intervals for cyanuric acid and the reference test against Antimycin A, a piscicide. Ten percent mortalities were observed at 210 and 1,200 mg/l after 72 hours. These were considered aberrant values and were attributed to natural causes.

Acute Toxicity of Cyanuric Acid and a Reference Compound (Antimycin A) to Fathead Minnows (Pimephales promelas)

	LC_{50} in milligrams/liter		
Compound	24 hours	48 hours	96 hours
Cyanuric Acid	>2,100	>2,100	>2,100
Antimycin A	0.000070	0.000048	0.000044
	(0.000060-0.000082)**	(0.000040-0.000057)	(0.000038-0.000052)

*Bioassay conducted at 22 °C (±1.0), mean weight and length, 0.17 g and 20.9 mm.
** 95% confidence interval

- Deaths per dose:
210 mg/l (10% mortality or 1/10 fish)
370 mg/l (0% mortality or 0/10 fish)
650 mg/l (0% mortality or 0/10 fish)
1200 mg/l (10% mortality or 1/10 fish)
2100 mg/l (0% mortality or 0/10 fish)

- Table showing cumulative mortality: At 24, 48, and 96 hours

Concentration (mg/l)	Observed Mortality %		
	24 hours	48 hours	96 hours
Control	0	0	0
210	0	0	10*
370	0	0	0
650	0	0	0
1,200	0	0	10*
2,100	0	0	0

* =These were considered aberrant values and were attributed to natural causes.

- Lowest Test Concentration Substance Causing 100% Mortality: none caused 100% mortality
- Mortality of Controls: none
- Reference Substance: Antimycin A (for results see LC_{50} table)
- Any observations: Precipitates were noted in all concentrations.

CONCLUSION

Author's Comments: The 96-hour LC_{50} of cyanuric acid for the four day static fish toxicity study using the fathead minnows is >2,100 mg/l.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 203.

REFERENCES (Free Text):

Forbis, A.D. and Thompson, C.M.; Acute Toxicity of Cyanuric Acid (AB-78-1384330-2c) to Fathead Minnows (Pimephales promelas); Study Conducted by Analytical Biochemistry Laboratories, Inc., Columbia, MO 65205 for Monsanto Chemical Company; Project No. BN-78-377; September 29, 1978.

American Public Health Association, "Standard Methods for the Examination of Water and Wastewater", 14th edition, New York, 1975.

U.S. EPA, Committee on Methods for Toxicity Test with Aquatic Organisms: Ecol. Res. Ser. (C.E. Stephen Chairman), "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians", 660/3-75009, 1975.

10e

Acute Toxicity to Fish

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: The procedures used in this test were described in the American Public Health Association's "Standard Methods for the Examination of Water and Wastewater" (1975) and the U.S. EPA's "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians" (1975). Comparable to OECD Guideline 203 "Fish, Acute Toxicity Test".

• Type (Test Type): Static Acute Toxicity
• GLP (Y/N): No
• Year Study Performed: 1978
• Species/Strain/Supplier: Rainbow Trout/Salmo Gairdneri/Beitey's Resort in Valley, Washington
• Analytical Monitoring: The test concentrations were prepared by weighing appropriate amounts of cyanuric acid and transferring directly to the test chambers.
• Exposure Period (unit): 96 hrs (4 days)
• Statistical Methods: LC_{50} calculated according to the method of Litchfield and Wilcoxon, 1949.
• Remarks field for Test Conditions:
- Pretreatment: All test fish were held in culture tanks on a 16 hour photoperiod and observed for at least fourteen days prior to testing. During this period, the fish received standard commercial fish food (Rangen's) daily until 48 hours prior to testing at which time feeding was discontinued.
- Test Fish: mean weight 1.07 g; and standard length 43.9 mm
- Dilution Water Source: Laboratory well water
- Dilution Water Chemistry: Dissolved oxygen (8.6 mg/l; pH 7.8); total hardness 220 mg/l $CaCO_3$; and total alkalinity 210 mg/l $CaCO_3$
- Stock and Test Solution: Before the stock solution was prepared, the standard was allowed to warm to room temperature (22 ^{o}C). The test concentrations were prepared by weighing appropriate amounts of Cyanuric Acid and transferring directly to the test chambers.
- Concentrations: 210 to 2,100 mg/l, (A preliminary range finding test indicated that the toxicity of cyanuric acid exceeded 1,000 mg/l)
- Vehicle/solvent and concentrations: None
- Stability of the Test chemical solutions: not specified
- Exposure vessel: Five gallon glass vessels containing 15 liters of laboratory well water, kept in water bath at 12 ^{o}C (± 1)
- Number of replicates, fish per replicate: 10 fish per concentration
- Water Chemistry in test: Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.
- Test temperature range: The vessels were kept in a water bath at 12 ^{o}C (± 1).

RESULTS

• Nominal concentrations: 210, 370, 650, 1200, 2100 mg/l
• Measured Concentrations: Not reported
• Unit: mg/l (ppm)
• Element value: LC_{50} at 96 hours = >2,100 mg/l Cyanuric Acid
• Remarks field for Results: The dissolved oxygen concentration, which stayed between 60% and 100% saturation was considered adequate for testing. The pH values for all concentrations remained consistent with the controls throughout the test and the ammonia concentrations were below the toxic limit.

- Table Showing Cumulative mortality: At 24, 48, 72 and 96 hours

Concentration (mg/l)	no. dead/ total no.	Observed Mortality %
Control	0	0
210	0	0
370	0	0
650	0	0
1,200	0	0
2,100	0	0

- Lowest Test substance concentration showing 100% mortality: none
- Mortality of controls: there was no mortality of controls
- Reference substance: Antimycin A
- The results of the four day static fish toxicity study using rainbow trout are summarized below.

Compound	96-hour LC_{50} (95% C.I.)
Cyanuric Acid	>2,100 mg/l
Antimycin A	0.000066 mg/l (0.000056-0.000078 mg/l)

- Any observations (i.e. precipitation): Precipitates were noted at all concentrations.

CONCLUSION

Author's Comments: The observed 96 hour LC_{50} and 95% confidence limits for Antimycin A were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition.

DATA QUALITY

• Reliabilities: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 203.

REFERENCES (Free text):

Forbis, A.D. and Thompson, C.M.; Acute Toxicity of Cyanuric Acid (AB-78-1384330-2d) to Rainbow Trout (Salmo Gairdneri); Study Conducted by Analytical Biochemistry Laboratories, Inc., Columbia, MO 65205 for Monsanto Chemical Company; Project No. BN-78-377; September 29, 1978.

American Public Health Association, "Standard Methods for the Examination of Water and Wastewater", 14th edition, New York, 1975.

U.S. EPA, Committee on Methods for Toxicity Test with Aquatic Organisms: Ecol. Res. Ser. (C.E. Stephen Chairman), "Methods of Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians", 660/3-75009, 1975.

11a

Toxicity to Aquatic Plants

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No.: 87-90-1
• Remarks field for Test Substance: White granules

METHOD

• Method/Guideline followed: Modified ASTM E645-85 (Standard Test Method for Efficacy of Microbicides Used in Cooling Systems)
• Test Type: Static
• GLP (Y/N): Yes
• Year (Study Performed): 1990
• Species/Strain # and source:
Chlorella pyrenoidosa (GBL #A310; ATCC #*UTEX-26)
Euglena gracilis (GBL #A311; ATCC #*UTEX-753)
Scenedesmus obliguus (GBL #312; ATCC #*UTEX-1450)
• Element Basis (i.e. number of cells/ml): Not provided
• Exposure period: 10 days
• Analytical Monitoring: Water analyzed for chlorine
• Statistical Methods: None
• Remarks field for Test Conditions:
- Method of Cultivation:
Inoculum Preparation: Grown at 20-25 ^{o}C under bright diffuse daylight, in Algae broth for 10 days. A 1:10 dilution in sterile normal saline was made and 0.1 ml was used to inoculate sample.
Inoculum Recovery: Ten-fold serial dilutions were inoculated (1 ml + 9 ml) into Algae broth. The tubes were incubated at 20-25 ^{o}C under bright diffuse sun light for 10 days.
- Controls: 3 hour control count
- Test Temperature range: Incubated at 20-25 ^{o}C
- Growth Medium Chemistry: N/A
- Dilution Water Source: Chlorine demand-free deionized water
- Exposure Vessel Type: 100 mL glass jars kept in 15 ^{o}C water bath 2 hours prior to test. (See Test Design for additional information).
- Water Chemistry in Test: available chlorine: 97.6 mg/L in water sample
- Stock solutions preparation (vehicle, solvent, concentrations): 110.2 mg of test compound was added to approximately 500 ml chlorine demand-free deionized water in a 100 ml volumetric flask. The suspension was stirred in a magnetic stirrer until dissolved and then diluted to 1000 ml with the sample mixture. The water sample was analyzed for chlorine.
- Light Levels: Bright diffuse sunlight
- Test design (number of replicated, concentrations):
Test #1: 5.0 ml of stock solutions was added to 100 ml sterile chlorine demand free deionized water (i.e., 5 mg/L)
Test #2: 1.0 ml of stock solution was added to 100 ml sterile chlorine demand free deionized water (i.e., 1 mg/L)
Test #3: 0.5 ml of stock solution was added to 100 ml sterile chlorine demand free deionized water (i.e., 0.5 mg/L)
Following three hours contact time, a 100 ml test samples was filtered through a 0.45 micron membrane filter. The filter was then rinsed with 100 ml sterile 0.1% peptone water. Each membrane filter was then transferred to 100 ml Algae broth in an eight ounce jar and mixed. Ten-fold serial dilutions were then poured into 9 ml Algae Broth. The Algae broth jars were incubated at 20-25 ^{o}C under bright diffuse sunlight for 10 days. After incubation, the jars and tubes were checked microscopically for growth.
- Method of calculating mean measured concentrations: Not measured

RESULTS

• Nominal Concentrations: 0.5, 1, 5 mg/L
• Measured Concentrations: Not determined
• Unit: mg/l (ppm) mg/L
• Element Value: EC_{50} at 72 and 96 hours EC_{90} after 3 hours: 0.5 mg/L test compound for C. pyrenoidosa, E. gracilis and S. obliguus
• NOEL: <0.5 mg/L
• LOEL: <0.5 mg/L
• Was control response satisfactory: Yes
• Statistical Results: None
• Remarks field for Results:

SUMMARY OF ACTIVATION RESULTS FOR ALL STRAINS:

Strain	Test #1 (5.0 mg/L)	Test #2 (1.0 mg/L)	Test #3 (0.5 mg/L)
Chlorella pyrenoidosa	Total Inactivation	Total Inactivation	Total Inactivation
Euglena gracilis	Total Inactivation	Total Inactivation	Total Inactivation
Scenedesmus obliguus	Total Inactivation	Total Inactivation	Total Inactivation

CONCLUSIONS

Author's Comments: 0.5 mg/l of test compound, following a three hour contact time, killed greater than or equal to 90% of Euglena gracilis, Scenedesmus obliguus and Chlorella pyrenoidosa.

DATA QUALITY

• Remarks field for Data Reliability: This study was not performed in accordance with standard OECD or EPA guidelines; however, since there was total inactivation within 3 hours, the methods and results are sufficient for evaluating the test compounds.

REFERENCES (Free text):

Prince, D. L., Efficacy of Towerchlor 90 Chlorine Demand Free Sterile Deionized Water Samples Containing Algae, Bacteria and Fungi, Gibraltar Biological Laboratories, Inc., Study No. GR298, Report No. 56104, Nov. 14, 1990.

11b

Toxicity to Aquatic Plants

TEST SUBSTANCE

• Identity: Cyanuric acid; CAS No. 108-80-5
• Remarks field for Test Substance:
- Purity: 77.5%; White, coarse powder.
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: Culture and test procedures followed similar to those of the U.S. Environmental Protection Agency (1971) (Algal Assay Procedure: Bottle Test)
• Test Type: Phytotoxicity Test
• GLP (Y/N): No
• Year (Study Performed): 1978
• Species/Strain # and source: Freshwater green alga, Selenastrum capricornutum (The culture was obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory, Corvallis, Oregon)
• Element Basis (i.e. number of cells/ml): not specified
• Exposure period: 96 hours
• Analytical Monitoring: Test concentrations were prepared by adding appropriate weighed amounts of test material to each flask.
• Statistical Methods: Each test concentration was converted to a logarithm and the corresponding percentage decrease of in vivo chlorophyll or cell numbers was converted to a probit (Finney, 1971). The 24-, 48-, 72-, and 96-hour EC_{50}'s and 90% confidence interval were then calculated by linear regression.
• Remarks field for Test Conditions:
- Test Organisms: method of cultivation: cultures were incubated at 24±1 ^{o}C under 4,000 lux illumination; controls: triplicate cultures were employed for the control.
- Test Temperature range: 24±1 ^{o}C
- Light Levels: 4,000 Lux illumination
- Test design: Triplicate cultures were employed for each of the test concentrations and the control.

RESULTS

• Nominal Concentrations: 56, 100, 320, 560, and 1,000 mg/l (based on a 96 hr range finding study)
• Measured Concentrations: Not reported
• Unit: mg/l (ppm)
• Element Value: EC_{50} at 72 and 96 hours
The estimated 24-hour and 48-hour EC_{50}'s based on decrease of in vivo chlorophyll a, were >1,000 ppm while the calculated 72- and 96-hour EC_{50}'s were 872 and 712 ppm respectively. Based on decrease of cell numbers, the 96-hour calculated EC_{50} was 655 ppm with 95% confidence limits of 439-977 ppm.
• NOEL: 48 hours
• LOEL: 72 hours
• Was control response satisfactory: yes
• Statistical Results: Growth of cultures exposed to concentrations of < or = to 100 ppm was enhance throughout the 96 hours of exposure. After 96 hours of exposure, decrease of in vivo chlorophyll a was from 14% in cultures exposed to 320 ppm to 66% in cultures exposed to 1,000 ppm, and decrease of cell numbers was from 15% in cultures exposed to 320 ppm to 76% in those exposed to 1,000 ppm.

• Remarks field for Results:

Effect Criterion	Hour	EC_{50} (mg/l; ppm)	95% confidence limits (mg/lppm)
In vivo Chlorophyll a	24	>1,000	---
	48	>1,000	---
	72	872	313-2,428
	96	712	424-1,198
Cell number	96	655	439-977

- Cell density in each flask:

			Percentage Change				
Concentration (mg/l; ppm)	pH		Chlorophyll a				Cell No.
	0 h	96 h	24 h	48 h	72 h	96 h	96 h
Control	7.9	8.0	---	---	---	---	---
56	7.9	8.0	+ 7	+ 33	+ 2	+ 9	+ 3
100	7.9	8.0	+ 2	+ 33	+ 5	+ 8	+ 4
320	7.8	8.0	+ 2	+ 16	- 19	- 14	- 15
560	7.7	7.8	- 2	- 3	- 43	- 42	- 39
1,000	7.6	7.8	- 5	- 16	- 52	- 66	- 76

- Growth Curves: the chart above shows the growth trends
- Observations: Cell counts were made using a hemacytometer and a Zeiss Standard 14 compound microscope. Measurements of in vivo chlorophyll in culture were performed by using a Turner Model III Fluoremeter.

CONCLUSIONS

Author's Comments: Exposure to cyanuric acid did not appear to adversely affect Selenastrum capricornutum until after 72 hours of exposure.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: OECD guideline 201 "Alga, Growth Inhibition Test" is most relevant with regard to method/design.

REFERENCES (Free text):

Hollister, T.A.; Acute Toxicity of Cyanuric Acid (BN-78-1384330-1) to the Fresh-Water Alga Selenastrum Capriocornutum; Study by EG&G Bionomics, Marine Research Laboratory, Pensacola, FL 32507 for Monsanto Industrial Chemicals Company; Report No. BP-78-9-137; Study No. H74-500. September 1978.

12a

Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: white powder

METHOD

• Method/Guideline followed: "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians," EPA, 1975 (EPA-660/3-75-009)
• Test type: Static bioassay
• GLP (Y/N): Unknown
• Year (study performed): 1976
• Analytical Procedures: N/A
• Species/Strain: *Daphnia magna*
• Test Details: Static
• Statistical Methods: Test concentrations and corresponding average observed percentage mortalities were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50 values and their 95% confidence intervals were calculated from the regression equation.
• Remarks field for Test Conditions:
- Test Organisms: Organisms from laboratory stocks cultured at EG&G Bionomics, Wareham, Massachusetts
- Stock solutions preparation: (vehicle, solvent, concentrations) and stability: The static bioassay was conducted in 250 ml beakers which contained 150 ml of test solution. For each concentration, the appropriate amount of test compound, dissolved in acetone, was pipetted into 500 ml of diluent water and mixed with a magnetic stirrer. A negative control, consisting of the same dilution water and conditions, but with no test compound, was established. A positive control was also established utilizing the same conditions plus the greatest amount of acetone present in any test vessel.
- Test temperature range: 22 ^{o}C +/- 1
- Exposure Vessel: No aeration
- Dilution Water Source: Well water
- Dilution Water Chemistry: Hardness: 35 mg/L as $CaCO_3$; pH: 7.1; DOC: greater than 60% of saturation
- Water Chemistry in Test: pH during 48h: 7.4-7.7; Dissolved Oxygen during 48h: 6.2-7.8 mg/L
- Element (unit) basis (*i.e.*, immobilization): mortality
- Test Design (# of replicates, individuals per replicate, concentrations (static/semistatic)): three 150 ml aliquots were divided among three beakers; five Daphnids assigned to each test vessel; 15 Daphnids per concentration

RESULTS

• Nominal Concentrations: 0.10, 0.16, 0.24, 0.37 and 0.56 mg/L
• Measured Concentrations: N/A
• Unit: mg/L
• Element value: 24h LC50: 0.34 mg/L (0.21-0.54 mg/L); 48h LC50: 0.21 mg/L (0.15-0.28 mg/L); NOEC at 48h: 0.10 mg/L
• Statistical Results: 95% confidence intervals
• Remarks field for Results:
- Number immobilized as compared to the number exposed:

Nominal Concentration (mg/L)	24h Percent Mortality	48h Percent Mortality
Control	7	7
Control (acetone)	0	7
0.10	0	0
0.16	0	13
0.24	0	20
0.37	93	100
0.56	100	100

- Was control response satisfactory: Yes

CONCLUSIONS

Author's Comments: The highest nominal concentration of the test compound at which there was no discernible effect on the test animals during the 48-hour bioassay was 0.10 mg/L.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: deviations from guideline include (a) 15 Daphnids per concentration were exposed rather than required minimum of 20 (40CFR797.1300.c.4.ii); (b) test Daphnids were less than or equal to 24 hours old, however, Guidelines require Daphnids to be acclimatized in test water for at least 48 hours prior to start of test (40CFR1300.d.1.ii); and, (c) no indication of using photoperiod of 16 hours light and 8 hours darkness (40CFR1300.d.1.iv).

REFERENCES (Free text):

Acute Toxicity of ACL-85 to Daphnia magna, EG&G, Bionomics Aquatic Toxicology Laboratory, Report No. BN 76-163, submitted to Monsanto Co., November 1976; MRID 00019383.

REMARKS FIELD FOR GENERAL REMARKS:

CYANURIC ACID - Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the environment because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products. The following data provides information on cyanuric acid and is provided here as support s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1.

CYANURIC ACID RESULTS -

(a) LC50 for 24h and 48h >1,000 mg/l; Nominal Concentrations: 0, 100, 130, 220, 360, 600, 1,000 mg/l. Cumulative mortality shown in table below.

Cyanuric Acid	Average Percentage Mortality (n=15)	
Nominal Concentration (mg/l)	24-hour	48-hour
1,000	0	0
600	0	0
360	0	0
220	0	0
130	0	0
100	0	0
Control	0	0

(b) LC50 at 24h: 8800 (6600-12,000) mg/l; LC50 at 48h: 6000 (5000-7000) mg/l; NOEL at 48 hrs 3200 mg/l; Nominal concentrations: 560, 1000, 1800, 3200, and 5600 mg/l. Cumulative mortality is shown in tables below.

At 24 hours

Concentration (mg/l)	no. dead/ total no.	Observed Mortality %	Expected Mortality %
Control	0/20		
1800	0/20	0	0
3200	0/20	0	1.4
5600	3/20	15	16

At 48 hours

Concentration (mg/l)	no. dead/ total no.	Observed Mortality %	Expected Mortality %
Control	0/20	0	
1800	0/20	0	0
3200	0/20	0	1.4
5600	8/20	40	40

REFERENCES:

LeBlanc, Gerald A.; Acute Toxicity of Cyanuric Acid to the Water Flea; Study Conducted by EG&G Bionomics, Aquatic Toxicology Laboratory, Wareham, MA for FMC Corporation; ICG/T-78-076; October 1977.

McAllister, W.A. and Thompson C.M.; Acute Toxicity of Cyanuric Acid (AB-78-1384330-2a) to Daphnia Magna; Study Conducted by Analytical Biochemistry Laboratories, Columbia, MO 65205 for Monsanto Chemical Company; Project No. BN-78-377A; September 30, 1978.

Health Elements

13.1a

Acute Oral

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method/guideline followed (experimental/calculated): Comparable to OECD Guideline 401 "Acute Oral Toxicity".
• Type (test type): Acute Oral Toxicity
• GLP (Y/N): Not identified on EPA Data Evaluation Record
• Year (study performed): 1985
• Species/Strain: Rat/Sprague Dawley (Charles River)
• Sex: Male/female
• No. of animals per sex per dose: 5/sex/dose
• Vehicle: Mineral Oil
• Route of administration: Oral, gavage
• Remarks field for Test Conditions:
- Age: 200-300 gms
- Doses: 0, 250, 500, 750, 1000, 2000 mg/kg_{bw}
- Doses per time period: One
- Post dose observation period: 14 days

RESULTS

• Value [LD_{50} or LC_{50}] with confidence limits if calculated:
Male LD_{50} (mg/kg_{bw}) 787 (95% confidence limits: 585,1059)
Female LD_{50} (mg/kg_{bw}) 868 (95% confidence limits: 622,1114)
Combined LD_{50} (mg/kg_{bw}) 809 (95% confidence limits: 692,927)
• Number of deaths at each dose level:

	#dead / #dosed	
Dose (mg/kg_{bw})	Males	Females
0	0/5	0/5
250	0/5	0/5
500	0/5	1/5
750	2/5	2/5
1000	5/5	3/5
2000	5/5	5/5

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 401. The EPA Data Evaluation Record classifies this study as "Guideline".

REFERENCES (Free text):

"Acute Oral Toxicity in Rats Trichlorocyanuric Acid", Final Report; Hazleton Laboratories America, Project No. 2291-100; February 5, 1985.

EPA Data Evaluation Record, March 26, 1987, MRID 150959.

13.1b

Acute Oral

TEST SUBSTANCE

• Identity: Cyanuric acid (crude); CAS No. 108-80-5
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed (experimental/calculated): Comparable to OECD Guideline 401 "Acute Oral Toxicity"
• Type (test type): Acute Oral Toxicity
• GLP (Y/N): Yes
• Year (study performed): 1981
• Species/Strain: Rat/Sprague Dawley
• Sex: Male/female
• No. of animals per sex per dose: 5/sex
• Vehicle: Water
• Route of administration: Oral, gavage
• Remarks field for Test Conditions:
- Age: 249-276 gms (males)/181-202 gms (females)
- Doses: 5,000 mg/kg_{bw}
- Doses per time period: One
- Volume administered or concentration: 397 mg/ml
- Post dose observation period: 14 days

RESULTS

• Value: [LD_{50} or LC_{50}] with confidence limits if calculated: Male LD_{50} (mg/kg_{bw}) >5,000; Female LD_{50} (mg/kg_{bw}) >5,000
• Number of deaths at each dose level: None
• Remarks field for Results:
- Time of death: No deaths
- Clinical Signs: Clinical abnormalities were observed in two animals, one of each sex. Lack of fecal material was observed in both of these animals and sedation, ataxia, piloerection, urine stained fur, porphyrin around the nose and body, weight loss also occurred in the male. With the exception of body weight loss, which occurred during the first week of the study, these effects had all subsided by the fourth day after dosing.
- Necropsy findings: Hydrometra was observed in one female rat, not considered related to test material by the study directors.
- If both sexes tested, results should be compared: LD_{50} >5,0000 mg/kg for both male and females

CONCLUSIONS

No deaths followed a single oral dosage of 5,000 milligrams per kilogram (mg/kg) of crude cyanuric acid to fasted albino rats of both sexes. Therefore, the acute oral LD50 of this material is considered to be in excess of 5,000 mg/kg.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study was conducted under GLP and with minor exceptions is similar in method/design to OECD guideline 401.

REFERENCES (Free text):

"Acute Oral Toxicity of Crude Cyanuric Acid to Rats", Monsanto Company Env. Health Laboratory, St. Louis, Missouri, EHL Study No. 810050, October 22, 1981.

13.2a

Acute Inhalation

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method/Guideline followed: Comparable to OECD Guideline 403 "Acute Inhalation Toxicity".
• Type: Acute Inhalation Toxicity
• GLP: Yes
• Year: 1985
• Species/Strain: Rats/Sprague Dawley (Charles River)
• Sex: Male/female
• No. of Animals per sex per dose: 10/sex/dose
• Vehicle: Room air (The test atmosphere was dust generated by passing a stream of air through the test articles contained in a dust shaker mechanism.)
• Route of Administration: Inhalation, whole body
• Remarks field for Test Conditions:
- Age: Per report "young adult"
- Doses: 0.29, 0.09 mg/l (gravimetric concentration)
- Doses per time period: One
- Particle size: Mean mass median diameter: 2.78 micrometers (geometric standard deviation 2.40)
- Post Dose Observation Period: 14 days
- Exposure Duration: 4 hours

RESULTS

• LC_{50} Value: Combined LC_{50} (mg/l) >0.09; <0.29 mg/l
• Number of deaths at each dose level:

Gravimetric Concentration (mg/l)	Mortality	
	Males	Females
0.29	3/5	3/5
0.09	0/5	0/5

• Remarks field for Results:
- Time of Death: Three males and 3 females died in the 0.29 mg/l exposure group before the scheduled termination. Of the 3 males: 1 died 9 days post-dose, 1 died 1 day post-dose, and 1 died shortly after exposure. Of the 3 females: 1 died shortly after exposure and 2 died 2 days after exposure.
- Clinical signs: Salivation, squinting, prostration, lethargy, crusty eye, crusty muzzle, crusty nose, alopecia, lacrimation, yellow/brown stained fur, irregular breathing, gasping and poor coat quality were observed during the study. Most effects observed in rats in the 0.29 mg/l exposure group occurred during exposure to the air-dust mixture, and remained for the surviving rats throughout the exposure and post-dose observatory periods. Animals in the 0.09 mg/l exposure group had many of the same symptoms, but only during the post exposure period. Nine of the 14 surviving animals exhibited body weight gains during the investigational period.
- Necropsy findings: Two males and two females in the 0.09 mg/l group had no gross lesions. 1 male in the 0.29 mg/l group had no gross lesions. The study directors noted no significant differences in findings between the sexes. All effects were not observed in each animal. All of the rats that died before the scheduled termination had lung effects. Rats exposed at 0.09 mg/l had the following lesions: multiple scabs, alopecia (head, back, inguinal region, around eyes) of the skin and a spleen adhered to a stomach. Rats exposed at 0.29 mg/l had the following lesions: emphysema or discoloration of the lungs, hemorrhage/red mottling on all lobes of the lung; congestion of the liver; gaseous dilatation of the gastrointestinal tract; subcutaneous hemorrhage of the tail; pale bilateral kidneys; crusting and erosions on the feet; red nasal passages; discolored and/or crusted skin on the feel, skin and back.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 403.

CONCLUSIONS

Study Director's Comments: Under the conditions tested, the LC_{50} for Trichloroisocyanuric would be less than 0.29 mg/l but greater than 0.09 mg/l.

REFERENCES (Free text):

Dudek, B.R.; Four Hour Dust Inhalation Toxicity Study in Rats of Trichloroisocyanuric; Study Conducted by Toxigenics, Inc., Decatur, IL 62526 for Pazianos & Associates, Inc.; Study No. 420-1798; January 23, 1985.

13.3a

Acute Dermal

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: 99% pure, Off-white granular material.

METHOD

• Method/Guideline followed: Comparable to OECD Guideline 402 "Acute Dermal Toxicity".
• Type: Acute Dermal Toxicity
• GLP: Yes
• Year: 1988
• Species/Strain: Rabbits/New Zealand White
• Sex: Male/female
• No. of Animals per sex per dose: 5/sex/dose
• Vehicle: Tap water
• Route of Administration: Dermal
• Remarks field for Test Conditions:
- Age: 2000-3000 g
- Dose: 2000 mg/kg_{bw}
- Doses per time period: One
- Post Dose Observation Period: 9 days (terminated prior to 14 days for humane reasons due to severe corrosive effects.
- Exposure Duration: 7 hours (occluded) For humane reasons, the exposure period was terminated 7 hours after application due to severe corrosive effects.

RESULTS

• Value:
Male LD50 (mg/kg_{bw}) >2000
Female LD50 (mg/kg_{bw}) >2000
• Number of deaths at each dose level: None
• Remarks field for Results:
- Clinical Signs: A variety of toxicological signs were observed in the hours after application, which included depression, rapid respiration, labored respiration, and phonation at the time of compound removal. The animals regained a normal appearance, except for dermal effects, from Day 1 to 9. Erythema (Grade 4) was seen in all of the rabbits on Day 1 Erythema (Grades 1-3) persisted in all the animals until termination on Day 9. Edema (Grades 1-2) was observed in all the animals on Day 1 and persisted as Grades 1 or 2 until termination. Other dermal effects included necrosis, fissuring with bleeding, and sloughing.
- Necropsy findings: Necropsy findings involved the spleen (enlarged) and kidneys (discoloration) and the treated skin, which exhibited necrosis in all of the rabbits.

Observation	Males	Females
No. Observed	5	5
No. with no gross lesions	0	0
Kidneys – pale	1	2
Spleen – enlarged	0	1
Skin - necrosis at application site	5	5

CONCLUSIONS

Study Director's Comments: The study was terminated prior to protocol-specified 14 days for humane reasons. It is believed that the corrosivity, and the absence of dermal toxicity have been established by the study despite these deviations from the protocol.

No deaths occurred during the study. Based upon the results of this study, the acute dermal LD50 of Biocare 90 is estimated to be greater than 2000 mg/kg of body weight in male rabbits, female rabbits and male and female rabbits combined.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 402.

REFERENCES (Free text):

Lemen, J.; Acute Dermal Toxicity Study in Rabbits; Study Conducted by Hazleton Laboratories America, Inc., Vienna, VA 22180 for Fertilizers & Chemicals, Ltd., Israel; Hazleton Study No. 2514-101; April 12, 1988. MRID 40835802.

13.3b

Acute Dermal

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method/guideline followed (experimental/calculated): Comparable to OECD Guideline 402 "Acute Dermal Toxicity".
• Type (test type): Acute Dermal Toxicity
• GLP (Y/N): Not identified on EPA Data Evaluation Record
• Year (study performed): 1984
• Species/Strain: Rat/Sprague Dawley (Charles River)
• Sex: Male/female
• No. of animals per sex per dose: 5/sex/dose
• Vehicle: Acetone
• Route of administration: Dermal
• Remarks field for Test Conditions:
- Age: 225-300 gms
- Dose: 5000 mg/kg_{bw}
- Doses per time period: One
- Post dose observation period: 14 days

RESULTS

• Value [LD_{50} or LC_{50}] with confidence limits if calculated:
Male LD_{50} (mg/kg_{bw}) >5,000
Female LD_{50} (mg/kg_{bw}) >5,000
• Number of deaths at each dose level: None
• Remarks field for Results:
- Clinical Signs: Slight depression was observed in all rats 4 hours post dose and on days 1 and 2, and in one or more rats to day 14. All rats were normal on day 14.

CONCLUSIONS

N/A

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 402. The EPA Data Evaluation Record classifies this study as "Guideline."

REFERENCES (Free text):

Gargas, J., "Acute Dermal Toxicity Study in Rats Trichlorocyanuric Acid (Trichloro-s-triazinetrione)", Final Report; Hazleton Laboratories America, Project No. 2291-101; August 13, 1984.

EPA Data Evaluation Record, March 26, 1987, MRID 150960.

13.3c

Acute Dermal

TEST SUBSTANCE

• Identity: Cyanuric Acid; CAS No. 108-80-5
• Remarks field for Test Substance:
- The appearance of the substance is off-white solid balls.
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: Comparable to OECD Guideline 402 "Acute Dermal Toxicity"
• Type: Acute Dermal Toxicity
• GLP: Yes
• Year: 1981
• Species/Strain: Rabbits/New Zealand White (Isaac's Farm, Litchfield, Illinois)
• Sex: Male/female
• No of Animals/sex/dose: 5/sex/dose
• Vehicle: Physiological saline
• Route of Administration: Dermal
• Remarks field for Test Conditions:
- Age: 2.51-3.79 kgt
- Dose: 5,000 mg/kg_{bw} (Occluded)
- Doses per time period: One
- Post Dose Observation Period: 14 days
- Exposure duration: 24 hours

RESULTS

• Value: LD_{50} (mg/kg_{bw}) >5000 or both male and female sexes.
• Number of deaths at each dose level: None
• Remarks field for Results:
- Necropsy findings: One male animal had pale colored kidneys, one female animal had kidneys with mottled exteriors, and another female had tapeworm cysts in the mesentery. None of these effects were attributed to toxicity of the test material by the study directors.

CONCLUSIONS

Study Director's Comments: No deaths followed a dermal application of 5,000 milligrams per kilogram (mg/kg) of crude cyanuric acid to the shaved and abraded dorsal surface of albino rabbits of both sexes. Therefore, the acute dermal LD_{50} of this material is considered to be in excess of 5,000 mg/kg. No signs of toxicity of the test material were observed.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 402.

REFERENCES (Free text):

Branch, D.K.; Acute Dermal Toxicity of Crude Cyanuric Acid to Rabbits; Study Conducted by Monsanto Company, St. Louis, MO 63110 for Monsanto Industrial Chemicals Company; Study No. 810051/ ML-81-110; October 14, 1981.

13.4a

Dermal Irritation

TEST SUBSTANCE

• Identity: s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1

METHOD

• Method/guideline followed (experimental/calculated): Comparable to OECD Guideline 404 "Acute Dermal Irritation/Corrosion".
• Type (test type): Acute Dermal Irritation
• GLP (Y/N): Not identified on EPA Data Evaluation Record
• Year (study performed): 1984
• Species/Strain: Rabbit/New Zealand White (Hazleton Dutchland)
• Sex: Male/female
• No. of animals per sex per dose: 3/sex/dose
• Vehicle: None
• Route of administration: Dermal
• Remarks field for Test Conditions:
- Dose: 0.5 gms
- Doses per time period: One
- Volume administered or concentration: Undiluted
- Post dose observation period: 21 days
- Exposure duration: 24 hours

RESULTS

• Value [LD_{50} or LC_{50}] with confidence limits if calculated: not stated
Male: not stated
Female: not stated
Draize Score: not stated
The EPA Data Evaluation Record concluded: "Compound is a very mild irritant".
• Number of deaths at each dose level: None
• Remarks field for Results:
- Clinical Signs: "Very slight" to "moderate" erythema and "very slight" to "slight" edema was observed at all sites at 32-60 minutes. All sites had cleared at 96 hours. Other dermal effects included compound adhering to the skin, thickening, blanching, necrosis, epidermal scaling, and raw areas. All areas were normal at 7 days.

CONCLUSIONS

From EPA Data Evaluation Report: "Very slight" to "moderate" erythema and "very slight" to "slight" edema was observed at all sites at 32-60 minutes. All sites had cleared at 96 hours. Other dermal effects included compound adhering to the skin, thickening, blanching, necrosis, epidermal scaling, and raw areas. All areas were normal at 7 days.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 404. The EPA Data Evaluation Record classifies this study as "Guideline."

REFERENCES (Free text):

"Primary Dermal Irritation Study in Rabbits, Trichlorocyanuric Acid (Trichloro-s-triazinetrione)", Final Report; Hazleton Laboratories America, Project No. 2291-103; August 16, 1984.

EPA Data Evaluation Record, March 26, 1987, MRID 150961.

13.5a

Eye Irritation

TEST SUBSTANCE

• Identity: Trichloro-s-triazinetrinone (trichloroisocyanuric acid); CAS No. 87-90-1

METHOD

• Method/guideline followed (experimental/calculated): 16CFR1500.42
• GLP (Y/N): unknown
• Year: 1976
• Species/Strain: New Zealand rabbits
• Sex: unknown
• No. of animals per sex per dose: 6 animals (sex unknown) all exposed to 0.1 g test compound
• Vehicle: direct application of 0.1 g of test compound into everted eyelid.
• Route of administration: ocular
• Remarks field for Test Conditions:
- Age: unknown
- Doses: 0.1 g test compound
- Volume administered or concentration: 0.1 g
- Post dose observation period: 24h, 48h, 72h, 4 days, 7 days
- The right eye of each test rabbit served as the experimental site and the left eye served as the control. The lower lid of each experimental eye was gently pulled from the surface of the eye and 0.1 g of the material was distributed in the everted eyelid. The eyes were irrigated with 25 ml of distilled water, 15 seconds following installation of the sample. Observations for the presence of injury to the cornea, iris and bulbar and palpebral conjunctivae were made 24h, 48h, 72h, 4 days and 7 days after administration of the test compound.

RESULTS

• Value [LD50 or LC50] with confidence limits if calculated: N/A
• Number of deaths at each dose level: N/A
• Remarks field for Results:
- Classification of the relative irritancy of the test material was made according to the following scale, utilizing the highest mean reading obtained at either 24, 48 or 72 hours after installation of the test material:
0.0 = non-irritating
0.1-10.0 = practically non-irritating
10.1-25.0 = mildly irritating
25.1-50.0 = moderately irritating
50.1 – 110.0 = extremely irritating
The mean scores obtained at each time interval were as follows:
24h = 66.67
48h = 73.83
72h = 89.17
4d = 81.00
7d = 72.33
- Moderate to severe corneal opacity, iritis and conjunctivitis was observed in all experimental eyes for the duration of the study.

CONCLUSIONS

Author's Comments: Under the conditions of this study, the test sample is considered to be a severe ocular irritant. The test sample was found to be extremely irritating to the eye of the albino rabbit.

DATA QUALITY

• Remarks field for Data Reliability: This study was not performed in accordance with standard OECD or EPA guidelines; however, since severe eye irritation occurred, the methods and results are sufficient for evaluating the test compounds.

REFERENCES (Free Text)

"Ocular Irritation Test with 15 Second Wash of CDB-90 (Trichloroisocyanurate)", Final Report; Prepared for FMC Corporation, Princeton, NJ; Study No. 1809. Submitted by Foster D. Snell, Inc., October 25, 1976.

13.5b

Eye Irritation

TEST SUBSTANCE

• Identity: Trichloro-s-triazinetrinone (trichloroisocyanuric acid); CAS No. 87-90-1
• Remarks field for Test Substance: white powder

METHOD

• Method/guideline followed (experimental/calculated): 16CFR1500.42
• GLP: unknown
• Year: 1977
• Species/Strain: albino rabbits
• Sex: unknown
• No. of animals per sex per dose: 6
• Vehicle: direct application of 0.1 g of test compound to eyelid.
• Route of administration: ocular
• Remarks field for Test Conditions:
- Age: young adult
- Doses: 0.1 g test compound
- Volume administered or concentration: 0.1 g test compound
- Post dose observation period: 1, 3, 24, 48 and 72 hours and 7 days
- The test procedure was modified as follows:
Group I: Six rabbits; eyes unwashed following instillation of test material
Group II: Six rabbits; eyes washed 60 seconds following instillation of test material
Group III: Six rabbits; eyes washed 20 seconds following instillation of test material

RESULTS

• Value [LD50 or LC50] with confidence limits if calculated: N/A
• Number of deaths at each dose level: none
• Remarks field for Results:
- Corneal, iridial and conjunctival effects were observed in all six animals for Groups I, II and III. These effects persisted throughout the 7-day observation period.

CONCLUSIONS

Author's Comments: The test compound is a severe eye irritant to the rabbit eyes when not followed by a washout 60 or 20 seconds after instillation of the test material.

DATA QUALITY

• Remarks field for Data Reliability: This study was not performed in accordance with standard OECD or EPA guidelines; however, since severe eye irritation occurred, the methods and results are sufficient for evaluating the test compounds.

REFERENCES (Free Text)

"Eye Irritation Test in Rabbits, Trichloroisocyanurate", Final Report; Food and Drug Research Laboratories, Inc., September 1, 1977.

13.5c

Eye Irritation

TEST SUBSTANCE

• Identity: Trichloro-s-triazinetrione (trichloroisocyanuric acid); CAS No. 87-90-1

METHOD

• Method/guideline followed (experimental/calculated): EPA OPP 81-4
• Test Type: Acute Eye Irritation - Rabbit
• GLP (Y/N): Y
• Year: 1995
• Species/Strain: New Zealand rabbits
• Sex: Male/female
• No. of animals per sex per dose: 3 animals/sex/dose
• Vehicle: direct application of 0.1 g of test compound into conjunctival sac
• Route of administration: ocular
• Remarks field for Test Conditions:
- Age: adult
- Doses: 0.1 g test compound
- Volume administered or concentration: 0.1 g
- Post dose observation period: 7 days
- The right eye of each test rabbit served as the experimental site and the left eye served as the control. The lower lid of each experimental eye was gently pulled from the surface of the eye and 0.1 g of the material was distributed in the conjunctival sac. The eyes were irrigated with 30 ml of physiological saline, 20-30 seconds following installation. Ocular irritation was evaluated 1 h, 24 h, 48 h, 72 h, 4 days and 7 days after administration of the test compound. Fluorescein dye evaluation method was used at 24 hours and as needed to evaluate the extent of corneal damage.

RESULTS

• Value [LD50 or LC50] with confidence limits if calculated: N/A
• Number of deaths at each dose level: N/A
• Remarks field for Results:
- Ocular irritation was evaluated in accordance with Draize, et al., J. Pharmacol. Exp. Ther., 1944, 82, 377-390. The scores were further classified by the system of Kay and Calandra, J. Soc. Cos. Chem., 1962, 13, 281-289.
- Other than the eye irritation noted, there were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.
- Severe to extreme irritation was evident in all treated eyes throughout the study. An increase in the severity of irritation was noted on day 7. The animals were euthanized on day 9 for humane reasons.

CONCLUSIONS

Author's Comment's: Based on the scoring system used, the test material is classified as extremely irritating to the washed eye when instilled as received.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)

REFERENCES (Free Text)

Wnorowski, G., "Primary Eye Irritation, ACL-90", PSL Report 3450, Product Safety Labs, Feb 22, 1995.

13.6a

Dermal Sensitization

TEST SUBSTANCE

• Identity: Trichloro-s-triazinetrinone (trichloroisocyanuric acid); CAS No. 87-90-1
• Remarks field for Test Substance:
Trichloro-s-triazinetrinone (trichloroisocyanuric acid): large white tablet
Trichloro-s-triazinetrinone (trichloroisocyanuric acid): off white granular solid

METHOD

• Method/guideline followed: OECD 406 and Commission Directive 96/54/EC Method B6 Acute Toxicity (Skin Sensitisation)
• GLP (Y/N): Yes
• Year (study performed):2000
• Species/Strain: albino Dunkin Hartley guinea pig
• Sex: Male
• No. of animals per sex per dose: ten males per dose; 5 males for control.
• Vehicle: liquid paraffin BP, distilled water, dimethylformamide, and Freund's Complete Adjuvant.
• Route of administration (if inhalation – aerosol, vapor, gas, particulate): dermal injection
• Remarks field for Test Conditions:
- Age: 8-12 weeks old
- Doses: Two phases were involved; an induction of a response by intradermal injection and topical application and a topical challenge of that response. The concentrations of the test material for the induction and challenge phases were selected as:
(1) Intradermal Induction: (a) 1:1 ratio of Freund's Complete Adjuvant plus distilled water; (b) 1% w/w formulation of test material in liquid paraffin BP; (c) 0.1% w/w formulation of test material in 3% dimethylformamide; (d) 1% w/w formulation of test material in 1:1 preparation of Freund's Complete Adjuvant plus distilled water; and, (e) 0.1% w/w formulation of test material in 1:1 preparation of Freund's Complete Adjuvant plus distilled water.
(2) Topical Induction: 1% w/w in distilled water.
(3) Topical Challenge: 2%, 1% and 0.5% w/w in liquid paraffin BP
Control animals received intradermal induction and topical inductions using an identical procedure as for the test animals, except that the test materials was omitted from the injections and patches.
- Doses per time period:
Intradermal Induction occurred at Day 0.
Topical Induction occurred at Day 7
Topical Challenge occurred at Day 21 (Day 20 for Study No. 1402/007)
- Volume administered or concentration:
0.1 ml for injections; a thick, even layer of test material was applied to Whatman No. 4 patch for topical applications.
- Post dose observation period:
Intradermal Induction: Approximately 24 and 48 hours after intradermal injections in the shoulder region of each animal, the degree of erythema at the test materials injection sites was evaluated.
Topical Induction: The occlusive dressing and patch was kept in place for 48 hours. The degree of erythema was quantified 1 and 24 hours following removal of the test materials patch.
Topical Challenge: The occlusive dressing and patch was kept in place for 24 hours. The degree of erythema was quantified approximately 24 and 48 hours following removal of the patch.

RESULTS

• Value [LD50 or LC50] with confidence limits if calculated: Not a skin sensitizer
• Number of deaths at each dose level: None
• Remarks field for Results:

- Description, severity, time of onset and duration of clinical signs at each dose level:
Note: Barely perceptible erythema (grade +/-) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. Furthermore, transient challenge reactions (those which do not persist for at least 48 hours) will not be attributed to contact sensitisation.
2% w/w in liquid paraffin BP: A hardened dark brown/black-coloured scab precluding evaluation of erythema and oedema was noted at the challenge sites of nine test and three control group animals at the 24 and 48-hours observations. No other skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.
1% w/w in liquid paraffin BP: A hardened dark brown/black-coloured scab precluding evaluation of erythema and oedema was confined to the challenge site of one test group animal at the 24 and 48-hour observations. This reaction was not attributed to contact sensitisation. Discrete patchy erythema was noted at the challenge site of one test group animal at the 24-hour observation. The reaction was not present at the 48-hour observation and was therefore not attributed to contact sensitization. Desquamation was noted at the challenge site of one test group animal at the 48-hour observation. No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hours observations.
0.5% w/w in liquid paraffin BP: No skin reactions were noted at the challenge sites of the control group animals at the 24 or 48-hours observations.

CONCLUSIONS

Author's Comments: Under conditions of the test, the test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin. The test material did not meet the criteria for classification as a sensitiser according to EU labeling regulations Commission Directive 93/21/EEC. No symbol or risk phrase required.

DATA QUALITY

• Reliabilities: Klimisch Code 1a (Reliable without restriction)

REFERENCES (Free Text)

"ACL 90 Chlorinating Tablets: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method." D.J. Allen, Safepharm Laboratories Limited, SPL Project Number: 1402/007. December 11, 2000.

"ACL 90XG Chlorinating Composition: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation Method." D.J. Allen, Safepharm Laboratories Limited, SPL Project Number: 1402/006. December 11, 2000.

14a

Genetic Toxicity in vivo or Genetic Toxicity in vitro (Chromosomal Aberrations)

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- Purity: 99.6 %
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 475 "Mammalian Bone Marrow Chromosome Aberration Test"
• Type: Genetic Toxicity
• GLP (Y/N): Yes
• Year (study performed): 1981
• Species: Rat
• Strain: Sprague Dawley (Simonsen Laboratories)
• Sex: Male
• Route of Administration: Oral, gavage
• Doses/concentration levels:
0, 0.04, 0.20, 1.0, 5.0 g/kg_{bw} (Range Finding Study)
0, 1.25, 2.50, 5.0 g/kg_{bw} (Definitive Study)
• Exposure Period: Not applicable
• Statistical methods: After all the slides had been scored they were decoded, and the data for each animal were pooled. For each animal, the mitotic index, the total number of chromosomal aberrations, the number of aberrations in each category, and the percentage of aberrant cells were determined. The mean number (frequency) of chromosomal aberrations per cell was determined by dividing the total number of aberrations observed for each treatment group by the total number of metaphase cells analyzed for that group, and the SEM was determined. Severely damaged cells were included when the mean numbers of chromosome aberrations per cell were calculated, but were excluded when the frequencies of different types of aberrations were calculated.
For each treatment group, data on aberrations per cell was checked for conformity to a Poisson distribution. If the data followed a Poisson distribution, the means for each group were subjected to a square root transformation. If the results did not conform to a Poisson distribution, a square-root transformation was performed on the numbers of chromosomal aberrations per cell and means for each group were calculated from the transformed data. Using the transformed data, the Student's t-test was performed to compare the frequency of chromosomal aberrations per cell in a treatment group with that of the negative control. Differences were considered statistically significant when $p < 0.05$.
• Remarks field for Test Conditions:
- Age at study initiation: 160 - 210 g
- No. of animals per dose: 4 (Range Finding Study); 10 (Definitive Study)
- Vehicle: 4% carboxymethyl cellulose
- Duration of test: 48 hours
- Frequency of treatment: Single dose
- Sampling times and number of samples: 24 hours (5 animals/dose) 48 hours (5 animals/dose)
- Control groups and treatment: Positive control - Triethylenemelamine (TEM); Negative control: 4% carboxymethyl cellulose
- Clinical observations performed (clinical pathology, functional observations, etc.): Mortality was observed daily. Body weights were obtained on the day of treatment and at the time of sacrifice.
- Organs examined at necropsy (macroscopic and microscopic): Bone marrow from femur
- Criteria for evaluating results: The mitotic index of femur marrow cells recovered from each animal was determined from 1000 cells per animal. Fifty cells in metaphase from each animal were analyzed for chromosomal aberrations.
- Criteria for selection of M.T.D: The highest dose that allowed animal survival and a mitotic index of at least 1.0% in bone marrow cells.

RESULTS

• Remarks field for Results:
- Mortality at each dose level by sex:
Dose-Range Finding Study: No mortality was observed at any of the doses tested.
Definitive Cytogenetic Study: No mortality was observed at any of the doses tested.
- Mutant/aberration/mPCE/polyploidy frequency, as appropriate:
Dose-Range Finding Study: The mean mitotic index at the highest dose was 3.36%. Thus, 5.0 g/kg was chosen as the maximum tolerated dose (MTD), 2.5 g/kg as the intermediate dose (1/2 MTD), and 1.25 g/kg as the low dose (1/4 HTD) for the definitive cytogenetic experiments for both the 24 and 48 hour treatment regimens.
Definitive Cytogenetic Study: In the 24 hour treatment regimen, comparison of the means resulting from the various endpoints showed that none of the doses of sodium cyanurate was significantly different from the negative control ($p > 0.5$). The mean for the positive control, triethylenemelamine at 0.275 mg/kg, however, was highly significant ($p < 0.05$).

CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM MALE RATS EXPOSED TO SODIUM CYANURATE BY ORAL GAVAGE: 24-HOUR EXPOSURE

	Negative Control	Low Dose (1.25 g/kg)	Mid Dose (2.50 g/kg)	High Dose (5.0 g/kg)	Positive Control (0.275 mg/kg TEM)
Number of animals	5	5	5	5	5
Mitotic index (%)	5.28 ± 0.79	5.74 ± 0.71	7.28 ± 0.94	6.49 ± 0.96	4.39 ± 0.60
Number of cells analyzed	250	250	238	239	238
Number (%) normal cells	231 (92)	231 (92)	224 (94)	227 (95)	159 (67)
Number (%) abnormal cells	19 (8)	19 (8)	14 (6)	12 (5)	79 (33)
Number of gaps per cell (mean ± SEM)	0.05 ± 0.004	0.07 ± 0.008	0.04 ± 0.003	0.03 ± 0.003	0.10 ± 0.008
Number (%) abnormal cells with:					
Chromosome deletions	1 (0.4)	1 (0.4)	1 (0.4)	0	8 (3.4)
Chromosome exchanges	0	0	1 (0.4)	1 (0.4)	4 (1.7)
Chromatid deletions	7 (2.8)	8 (3.2)	7 (2.9)	6 (2.5)	50 (21.0)
Chromatid exchanges	8 (3.2)	13 (5.2)	2 (0.8)	5 (2.1)	32 (13.4)
Aneuploidy	4 (1.6)	2 (0.8)	4 (1.7)	1 (0.4)	5 (2.1)
Polyploidy	0	1 (0.4)	1 (0.4)	0	0
Severe damage	0	0	0	0	3 (1.3)
Types of aberrations per cell:					
Overall frequency of aberrations (mean ± SEM)	0.08 ±0.02	0.08 ±0.02	0.07 ±0.02	0.05 ±0.02	0.89 ±0.12*
Chromosome deletions	0.004	0.004	0.004	0.000	0.040
Chromosome exchanges	0.000	0.000	0.004	0.004	0.020
Chromatid deletions	0.03	0.03	0.03	0.03	0.50
Chromatid exchanges	0.03	0.03	0.01	0.02	0.20

*Significantly different from control, $p < 0.05$.

For 48 hours' exposure, none of the doses of sodium cyanurate was significantly different from the control values ($p > 0.05$), whereas, the positive control group showed a significant increase in chromosomal aberration ($p < 0.05$).

CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM MALE RATS EXPOSED TO SODIUM CYANURATE BY ORAL GAVAGE: 48-HOUR EXPOSURE

	Negative Control	Low Dose (1.25 g/kg)	Mid Dose (2.50 g/kg)	High Dose (5.0 g/kg)	Positive Control (0.275 mg/kg TEM)
Number of animals	5	5	5	5	5
Mitotic index (%)	5.25 ± 0.21	5.31 ± 0.18	4.52 ± 0.18	4.37 ± 0.24	5.52 ± 0.24
Number of cells analyzed	239	250	250	226	250
Number (%) normal cells	224 (94)	229 (92)	224 (90)	210 (93)	110 (44)
Number (%) abnormal cells	15 (6)	21 (8)	26 (10)	16 (7)	140 (56)
Number of gaps per cell (mean ± SEM)	0.06 ± 0.008	0.05 ± 0.005	0.07 ± 0.005	0.04 ± 0.005	0.13 ± 0.009
Number (%) abnormal cells with:					
Chromosome deletions	1 (0.40)	0	0	0	9 (3.6)
Chromosome exchanges	1 (0.4)	0	0	0	2 (0.8)
Chromatid deletions	7 (2.9)	11 (4.4)	10 (4)	4 (1.7)	76 (30.4)
Chromatid exchanges	3 (1.3)	7 (2.8)	15 (6)	7 (3)	64 (25.6)
Aneuploidy	3 (1.3)	3 (1)	0	5 (2.2)	10 (4)
Polyploidy	2 (0.8)	3 (1)	1 (0.40)	0	0
Severe damage	0	0	0	0	25 (10)
Types of aberrations per cell:					
Overall frequency of aberrations (mean ± SEM)	0.07 ±0.02	0.11 ±0.03	0.12 ±0.03	0.09 ±0.02	2.36 ±0.70*
Chromosome deletions	0.004	0	0	0	0.05
Chromosome exchanges	0.004	0	0	0	0.01
Chromatid deletions	0.03	0.06	0.06	0.02	0.95
Chromatid exchanges	0.01	0.03	0.06	0.04	0.45

*Significantly different from control, $p < 0.05$.

CONCLUSIONS

Author's Comments: "In each treatment regimen, none of the doses tested produced means that were significantly different from that of the negative control group. The positive control compound, TEM (0.275 mg/kg) induced significantly higher levels of chromosomal aberrations. Therefore, we conclude that, under our experimental conditions, sodium cyanurate does not induce any significant chromosomal damage in rat bone marrow cells. Thus, it appears that sodium cyanurate does not result in chromosomal abnormalities different from control levels at the doses tested in this study."

EPA Data Evaluation Record:
Dose-Range-Finding Study: Mitotic indices ranged from 1.50 to 6.18 % and showed no compound-related effects. A dose of 5 gm/kg was considered the MTD.
Definitive Cytogenetic Study: At the 24 hour sacrifice, no mortality was observed at any dose tested. No compound-related effects were observed at any dose tested. The positive control showed an approximately ten-fold increase in aberrations compared to the untreated controls.
At the 48 hour sacrifice, no mortality was observed at any dose tested. No compound-related effects were observed at any dose tested. The positive control showed an approximately thirty-fold increase in aberrations compared to the untreated controls.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 475. Single sex with no justification, cells examined 50 instead of 100. The EPA Data Evaluation Record classifies this study as "Acceptable".

REFERENCES (Free text):

"An evaluation of the mutagenic potential of sodium cyanurate using the in vivo rat bone marrow cytogenic assay", SRI International, SRI Project LSC-2923 Task 2; December, 1981.

EPA Data Evaluation Record, February 19, 1987 MRID 00091031.

Hammond, Bruce G, Barbee, Steven J., Wheeler, Allan G, and Cascieri, Tito, "Absence of Mutagenic Activity for Monosodium Cyanurate," Fundamental and Applied Toxicology, Vol. 5, pp. 655-664 (1985).

15a

Genetic Toxicity in vitro (Gene Mutations)

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No.: 2624-17-1
• Remarks field for Test Substance:
- Purity: 99.6%
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable OECD Guideline 479 "In Vitro Sister Chromatid Exchange Assay in Mammalians
• Type: Mammalian cell
• System of testing: non-bacterial
• GLP: Yes
• Year: 1981
• Cell line: Chinese hamster ovary (CHO) cells, ATCC CCL 61, CHO-K1, proline-requiring from American Type Culture Collection, Rockville MD
• Metabolic activation: Species and cell type: Rat liver S-9; Quantity: One part S-9 to nine parts metabolic activation mixture; Induced or not induced: Arochlor 1254 induced.
• Concentrations tested: Cytotoxicity: 0, 8, 40, 200, 1000, 5000 ug/ml (1000 and 5000 ug/ml were suspensions); Sister Chromatid exchange: 0, 93.8, 187.5, 375, 750, 1500 ug/ml (all concentrations had crystalline material which failed to go into solution). Highest concentration chosen on basis of limited solubility in culture medium.
• Statistical Methods: The mean SCE frequency per cell observed by each cytogeneticist for each concentration of the test article and for the controls was determined by dividing the total number of SCEs observed by the number of cells analyzed. Similarly, the mean SCE frequency per chromosome was determined by dividing the number of SCEs observed by the number of chromosomes counted. Since it is assumed that the numbers of SCEs per cell and per chromosome follow a Poisson distribution, the mean was used as the estimate of variance in calculating standard errors.
These means then were evaluated by a one-way analysis of variance. The mean SCE frequencies per chromosome and per cell that each cytogeneticist observed for the same treatment group (variance within samples) were compared with the mean SCE frequencies observed for the other treatment groups and for the negative control (variance between samples). An F test was then performed to determine whether the between-sample variance (the effect of the test article) was significantly greater than the within-sample variance (the variance between scorers and samples in the same treatment group). The positive control was not included in these analyses.
• Remarks field for Test Conditions:
- Number of replicates: 2
- Frequency of Dosing: 21.5 hour incubation (without metabolic activation); 2 hour incubation (with metabolic activation)
- Positive and negative control groups and treatment:
Positive control without metabolic activation: ethylmethane sulfonate (EMS) 10^{-3} M
Positive control with metabolic activation: dimethylnitrosamine (DMN) 10^{-3} M
Negative control without metabolic activation: complete McCoy's 5a medium with 10^{-5} BrdU
Negative control with metabolic activation: complete McCoy's 5a medium with 10^{-5} BrdU and complete metabolic activation mixture with the S-9 fraction.
- Number of metaphases analyzed: 100 cells
- Solvent: McCoy's 5a complete medium with BrdU and complete metabolic activation mixture with the S-9 fraction.
- Criteria for evaluating results (e.g. cell evaluated per dose group): A test article was considered to have elicited a positive response in the SCE assay if it induced a concentration-related elevation above baseline SCE frequencies and if comparison by one-way analysis of variance of the SCE frequencies of the test concentrations of the test article and the negative controls produced a value of $p < 0.05$.

RESULTS

• Cytotoxic concentration:
With metabolic activation: No cytotoxicity was observed at any dose level
Without metabolic activation: No cytotoxicity was observed at any dose level
• Genotoxic effects:
With metabolic activation: No compound related effect was observed on sister chromatid exchange at any dose level
Without metabolic activation: No compound related effect was observed on sister chromatid exchange at any dose level
• Statistical results: A one-way analysis of variance was conducted on SCE frequencies for the five concentrations of monosodium cyanurate and the negative controls (with/without metabolic activation). The variance between treatment groups was not significantly greater than the variance within treatment groups.
• Remarks field for Results:
- The solubility of monosodium cyanurate in the BrdU media was found to be less than 750 ug monosodium cyanurate per milliliter of solvent. In the cytotoxicity evaluation with and without metabolic activation, at concentrations of 1000 and 5000 ug/ml, a suspension of the test article resulted. This solubility limit determined the test material concentrations that could be used in the definitive assay. In the definitive assay, a crystalline material failed to go into solution during dilution of test article in BrdU media at all concentrations (a suspension was formed rather than a true solution in each case). Additional material precipitated from the BrdU medium during exposure of CHO cells at 750 to 1500 ug/ml.
- Frequency of reversions/mutations/aberrations, polyploidy:

Sister Chromatid Exchange (SCE) Frequencies in Chinese Hamster Ovary (CHO) Cells Exposed to Monosodium Cyanurate for 21.5 Hours Without Metabolic Activation

Treatment	Cytogenicist *	No. of SCE's	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell † (Mean ± SE)	SCEs/ Chromosome † (Mean ± SE)
Negative Control	A	461	962	19.24 ± 0.85	9.22 ± 0.43	0.479± 0.022
(BrdU medium)	B	430	964	19.28 ± 0.81	8.60 ± 0.41	0.446 ± 0.022
Monosodium cyanurate (µg/ml)						
93.8 l	A	386	981	19.62 ± 1.03	7.72 ± 0.39	0.393 ± 0.020
	B	400	958	19.16 ± 0.77	8.00 ± 0.40	0.418 ± 0.021
187.5 ‡	A	400	997	19.94 ± 1.24	8.00 ± 0.40	0.401 ± 0.020
	B	338	955	19.10 ± 0.84	6.76 ± 0.37	0.354 ± 0.019
375 ‡	A	471	952	19.04 ± 0.83	9.42 ± 0.43	0.495 ± 0.023
	B	324	955	19.10 ± 0.91	6.48 ± 0.36	0.339 ± 0.019
750 ‡§	A	490	976	19.52 ± 0.99	9.80 ± 0.44	0.502 ± 0.023
	B	300	954	19.08 ± 0.75	6.00 ± 0.35	0.314 ± 0.018
1500 ‡§	A	460	977	19.54 ± 0.86	9.20 ± 0.43	0.471 ± 0.022
	B	334	959	19.18 ± 0.77	6.68 ± 0.37	0.348 ± 0.019
Positive Control	A	2267	975	19.50 ± 0.71	45.34 ± 0.95	2.325 ± 0.049
10^{-3} M ethyl methanesulfonate	B	1214	963	19.26 ± 0.90	24.28 ± 0.70	1.261 ± 0.036

* Each cytogeneticist analyzed 50 cells per sample.
† A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to monosodium cyanurate and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.
‡ A crystalline material failed to go into solution during dilution of the test article in BrdU media at these concentrations.
§ Additional material precipitated from the BrdU media.

Sister Chromatid Exchange (SCE) Frequencies in Chinese Hamster Ovary (CHO) Cells Exposed to Monosodium Cyanurate for 2 Hours With Metabolic Activation

Treatment	Cyto-geneti-cist*	No. of SCE's	No. of Chromosomes	Chromosomes/Cell (Mean ± SD)	SCEs/Cell † (Mean ± SE)	SCEs/ Chromosome † (Mean ± SE)
Negative Control (complete metabolic activation mixture)	A	529	953	19.06 ± 0.89	10.58 ± 0.46	0.555± 0.024
	B	568	992	19.84 ± 1.15	11.36 ± 0.48	0.573 ± 0.024
Monosodium cyanurate (µg/ml)						
93.6 ‡	A	600	949	18.98 ± 0.68	12.00 ± 0.49	0.632 ± 0.026
	B	550	974	19.48 ± 1.13	11.00 ± 0.47	0.565 ± 0.024
187.2 ‡	A	613	955	19.10 ± 0.93	12.26 ± 0.50	0.642 ± 0.026
	B	612	973	19.46 ± 0.95	12.24 ± 0.49	0.629 ± 0.025
375‡	A	476	961	19.22 ± 0.91	9.52 ± 0.44	0.495 ± 0.023
	B	567	971	19.42 ± 1.03	11.34 ± 0.48	0.584 ± 0.025
750 ‡ §	A	534	947	18.94 ± 0.68	10.68 ± 0.46	0.564 ± 0.024
	B	575	978	19.56 ± 1.15	11.50 ± 0.48	0.588 ± 0.025
1500 ‡ §	A	547	965	19.30 ± 0.76	10.94 ± 0.47	0.567 ± 0.024
	B	729	979	19.58 ± 1.01	14.58 ± 0.54	0.745 ± 0.028
Positive Control	A	1309	943	18.86 ± 0.97	26.18 ± 0.72	1.388 ± 0.038
	B	1667	978	19.56 ± 1.11	33.34 ± 0.82	1.704 ± 0.042

* Each cytogeneticist analyzed 50 cells per sample.
† A one-way analysis of variance comparing the SCE frequencies in CHO cells exposed to monosodium cyanurate and to the negative control indicated that the variance between treatment groups was not significantly greater than the variance within treatment groups.
‡ A crystalline material failed to go into solution during dilution of the test article in metabolic mixture at these concentrations.
§ Additional material precipitated from the metabolic activation mixture.

CONCLUSIONS

Study Director's Comments: "…under the conditions of our experiments, monosodium cyanurate did not induce SCEs in CHO cells with or without metabolic activation."

EPA Data Evaluation Record:
"The test compound was not cytotoxic at doses of 8 to 5000 ug/ml either without or with metabolic activation. The test compound had no effect on sister chromatid exchange at doses of 93.8 to 1500 ug/ml either without or with metabolic activation."

DATA QUALITY

• Reliability: Klimisch Code: 1b (Reliable without restriction; Comparable to guideline study)
• Remarks for Data Reliability: The EPA Data Evaluation Record classifies this study as "Acceptable"

REFERENCES (Free text):

"An evaluation of the effect of monosodium cyanurate on sister chromatid exchange frequencies in cultured Chinese hamster ovary cells", SRI International, Project LSC-2923, Task 1, November 1981.

EPA Data Evaluation Report, March 18, 1987, MRID 94882.

Hammond, Bruce G, Barbee, Steven J., Wheeler, Allan G, and Cascieri, Tito, "Absence of Mutagenic Activity for Monosodium Cyanurate," Fundamental and Applied Toxicology, Vol. 5, pp. 655-664 (1985).

15b

Genetic Toxicity in vitro (Gene Mutations)

TEST SUBSTANCE

• Identity: Cyanuric acid (sodium salt); CAS No. 2624-17-1
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 476 "In Vitro Mammalian Cell Gene Mutation Test". The protocol was essentially the same as recommended by Clive and Spector (Mutation Res., 31 (1975) 17-29). In order to determine the optimal dose levels for the definitive study, a preliminary toxicity test with/without S-9 activation was conducted. The test article was tested in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with and without metabolic activation by induced rat liver S-9. The cultures treated without activation were cloned over a range of concentrations which produced from 69% to 113% suspension growth, and the cultures receiving S-9 metabolic activation were cloned over a range of concentrations which produced from 94% to 105% suspension growth.
• Type: Mammalian cell forward gene mutation at the thymidine kinase (TK) locus
• System of testing: non-bacterial
• GLP: Yes
• Year: 1981
• Cell line: L5178Y mouse lymphoma cells
• Metabolic activation: Species and cell type: Rat liver S-9; Quantity: 4 ml S-9/incubation tube; Induced or not induced: Not induced
• Concentrations tested:
Toxicity test: 0.01, 0.1, 1.0, 10, 100, 1000, 10,000 ug/ml
With metabolic activation: 250, 500, 750, 1000, 1250, 1500, 1750, 2000 ug/ml
Without metabolic activation: 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000 ug/ml
• Statistical Methods: After the incubation period, both the TFT (BUdR) plates and the V.C. plates are scored for the total number of colonies per plate. Three counts per plate are made on an automatic colony counter, and the median count is recorded. The mutation frequency is determined by dividing the average number of colonies in the three TFT (BUdR) plates by the average number of colonies x 10^4 in the three corresponding V.C. plates and multiplying the quotient by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
• Remarks field for Test Conditions:
- Number of replicates: 3
- Frequency of Dosing: Single, 4 hr exposure
- Positive controls with/without metabolic activation: ethylmethane sulfonate (EMS) and 7,12 dimethylbenz(a)anthracene (DMBA)
- Number counts per plate: 3
- Solvent: The test article was essentially insoluble in all of the standard solvents compatible with the system except deionized, distilled water in which it was only partially soluble. The maximum achievable concentration, with water as the solvent was 200 ug/ml.
- Criteria for evaluating results (e.g. cell evaluated per dose group): After the incubation period, plates with/without a restrictive cloning medium are scored for the total number of colonies per plate. For each dose level, the mutation frequency was determined by dividing the average number of colonies in the plates containing the restrictive medium by the average number of colonies x 10^4 in the control plates (no restrictive medium) and multiplying the quotient by two. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.

RESULTS

• Cytotoxic concentration:
With metabolic activation: 200 ug/ml (limit of solubility in water) no toxicity; 2000 ug/ml (suspension): 7% reduction in growth
Without metabolic activation: 200 ug/ml (limit of solubility in water) no toxicity; 2000 ug/ml (suspension): 10% reduction in growth

• Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal):
With metabolic activation: No significant increase in the mutant frequencies of treated cultures over that of the solvent controls.
Without metabolic activation: No significant increase in the mutant frequencies of treated cultures over that of the solvent controls.

Cyanuric Acid

		Concentration (ug/ml)								
		100	250	500	750	1000	1250	1500	1750	2000
Induced Mutation Frequency										
	Without Activation	-0.2	-0.1	-0.1	-	0.0	0.0	0.1	-0.2	0.1
	With Activation	-	0.0	-0.2	-0.1	-0.1	-0.1	-0.2	0.0	-0.1

Positive Controls

		EMS			DMBA	
		Concentration (ug/ml)				
		0.5 ul/ml	1.0 ul/ml		5.0 ug/ml	7.5 ug/ml
Mutation Frequency		9.1	30.5		4.8	--

• Remarks field for Results: Precipitation concentration: 200 ug/ml was limit of solubility in water, 2000 ug/ml was achieved as a suspension in the S-9 mix.

CONCLUSIONS

Study Director's Comments: Under these test conditions, the test article is considered negative in this assay.

For the cultures treated both in the presence and absence of induced rat liver S-9, none of the cultures which were cloned exhibited mutant frequencies which were significantly greater than that of the corresponding solvent control cultures. The % Total Growth exhibited by the nonactivated cultures ranged from 67% to 106% and for the S-9 activated cultures it ranged from 82% to 126%.

The test article did not significantly increase the mutant frequencies of any of the treated cultures over that of the solvent controls. The material was tested to the limits of the assay and under these test conditions, was found to be relatively non-toxic and non-mutagenic in this system.

EPA Data Evaluation Record: Cyanuric acid produced no elevation in the rate of gene mutation over that of the solvent controls under the conditions of this assay, both with and without rat liver S-9 metabolic activation.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to OECD Guideline 476 "In Vitro Mammalian Cell Gene Mutation Test")
• Remarks field for Data Reliability: Lack of details on statistical analysis, size of colonies not reported. EPA Data Evaluation Record states: "There are no serious deficiencies seen in this study. This study was performed adequately in accordance with the current scientific literature."

REFERENCES (Free text):

"Evaluation of Test Article Cyanuric Acid (Sodium Salt) (MRI #582) For Mutagenic Potential Employing the L5178Y TK+/- Mutagenesis Assay", EG&G Mason Research Institute, Study No. 013-312-582-7, May 21, 1981.

EPA Data Evaluation Report, October 13, 1981, MRID 94883.

Hammond, Bruce G, Barbee, Steven J., Wheeler, Allan G, and Cascieri, Tito, “Absence of Mutagenic Activity for Monosodium Cyanurate,” Fundamental and Applied Toxicology, Vol. 5, pp. 655-664 (1985).

15c

Genetic Toxicity in vitro (Gene Mutations)

TEST SUBSTANCE

• Identity: Cyanuric Acid; CAS No. 2624-17-1
• Remarks field for Test Substance:
- 77.34% pure
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Test procedures used were comparable to those described by Ames et al. (1975). Comparable to OECD Guideline 471 "Bacterial Reverse Mutation Test.".
• Type: reverse mutation assay
• System of testing: Bacterial
• GLP: No
• Year: 1980
• Species/Strain: *Salmonella typhimurium* test strains TA98, TA100, TA1535, and TA1537
• Metabolic activation: Species and cell type: S-9 preparations from livers of male Sprague-Dawley rats and CD-1 mice; Quantity: 10 mL; Arochlor 1254-induced
• Concentrations tested: 10 mg, 3 mg, 1 mg, 0.2 mg, 0.04 mg, and 0.01 mg per plate
• Statistical Methods: Bartlett's test and t-test were used to indicate test values significantly greater than the control ($p \leq 0.01$). Further analysis (dose-response analysis) was only performed on strains and test conditions indicating at least one treatment greater than the control ($p = 0.01$).
• Remarks field for Test Conditions:
- Number of replicates: Three
- Positive controls: TA98, TA100:2-nitrofluorene, benzo(a)pyrene; TA1535: $NaNO_2$, 2-aminoanthracine, TA1537:, 9-aminoacridine, 2-aminoanthracine
- Negative control: Water solvent
- Solvent: DMSO (dimethylsulfoxide) and water
- Description of follow up repeat study: Both toxicity screens and spot tests were conducted before the definitive plate incorporation study was performed. The initial plate incorporation assay performed on only TA98 and TA100 demonstrated inappropriate responses with solvent controls for all test conditions except with strain TA98 with rat microsomal activation. This showed no treatments significantly greater than controls ($p = 0.01$). Upon retest using all four tester strains, TA98, TA100, TA1535, TA1537. The results of the *Salmonella* plate incorporation assay demonstrated no mutagenicity of the test sample to any of the four tester strains (TA98, TA100, TA1535, or TA1537), either with or without a rat microsomal activation system and at a concentration of 10 mg of sample per plate. The spot test indicated no mutagenicity of the test sample at a concentration of 25 mg per spot with tester strains TA98, TA100, and TA1537 either in the presence or absence of mammalian microsomal activation factors. Solvent controls and test values were above the normal range in the initial spot assay with strain TA1535. A rerun of the spot assay with strain TA1535 confirmed no mutagenicity under the conditions tested. Slight toxicity was noted to the lawns of strain TA1537 in the original spot test.
- Criteria for evaluating results: A positive response is indicated if three or more treatments on the initial test and/or retest (if performed) are significantly greater than control ($p \leq 0.01$) and if there is a significant positive dose-response for the initial test (or retest if performed).

RESULTS

• Cytotoxic concentration:
With metabolic activation: No cytotoxicity at concentrations ≤10 mg of sample per plate
Without metabolic activation: No cytotoxicity at concentrations ≤ 10 mg of sample per plate

	Revertants/plate (TA100 strain only)		
	Sample		
Sample Quantity, mg/plate	Stock #1	Stock #2	Solvent Controls
With S-9			
10	215	220	229, 129, 263
3	193		
1	243		
0.2	206		
0.04	175		
0.01	217		
Without S-9			
10	170	211	228, 192, 220
3	189		
1	271		
0.2	163		
0.04	228		
0.01	196		

• Genotoxic effects:
With metabolic activation: No genotoxic effects at concentrations ≤ 10 mg of sample per plate
Without metabolic activation: No genotoxic effects at concentrations ≤ 10 mg of sample per plate

RESULTS OF THE SPOT TEST FOR MUTAGENICITY (note: results from the retest assay)

	Revertants/Plate	
Strain	Sample	Solvent Control
With Rat S-9:		
TA98	31	43
TA100	149	220
TA1535	16	19
TA1537	13	10
With Mouse S-9:		
TA98	30	28
TA100	296	284
TA1535	33	25
TA1537	11	14
Without S-9:		
TA98	25	18
TA100	277	339
TA1535	17	19
TA1537	10	9

RESULTS OF PLATE INCORPORATION TEST
With Microsomal Activation:

	Revertants/Plate											
Amount/Plate(mg)	TA98			TA100			TA1535			TA1537		
10	36	35	24	256	212	225	23	9	11	12	10	1
3	29	52	62	296	228	257	13	18	21	1	10	5
1	38	23	33	246	290	264	19	12	27	8	7	11
.2	38	35	31	249	245	278	16	16	10	13	8	8
.04	26	24	34	196	271	295	13	18	16	14	7	11
.01	34	38	48	231	295	261	19	22	24	12	7	12
Controls												
Solvent	42	33	32	245	207	315	13	24	19	10	12	15
Negative	42	33	32	245	207	315	13	24	19	10	12	15
Positive	1003			1863			509			270		

RESULTS OF PLATE INCORPORATION TEST (continued)
Without Microsomal Activation:

	Revertants/Plate								
Amount/ Plate	TA100			TA1535			TA1537		
10	320	276	288	26	27	18	9	9	13
3	283	282	264	28	23	21	14	8	9
1	284	263	290	20	30	15	13	8	5
.2	261	331	309	33	20	17	5	8	13
.04	291	242	305	19	11	29	9	5	6
.01	242	302	346	20	19	18	7	6	9
Controls									
Solvent	233	226	283	27	22	24	7	12	5
Negative	233	226	283	27	22	24	7	12	5
Positive	534			503			721		

SUMMARY OF SPOT, TOXICITY, AND PLATE INCORPORATION TESTS

		Toxicity Test - **b**		Plate Incorporation Test - **c**	
Sample	Spot Test - **a**	With S-9	Without S-9	With S-9	Without S-9
80-1-14-220	-d	-e	-e	-d	-d

a – With rat S-9, mouse S-9 and no S-9, and 25 mg sample/disk
b – At 6 concentrations, 10 mg/plate the maximum, with TA100
c – At 6 concentrations, with and without rat liver S-9, with strains TA98, TA100, TA1535, and TA1537
d – No mutagenic response at concentration tested.
e – No toxic response at concentrations tested.

• Statistical Results: No statistically significant differences were found in any of the assays between control and test substance groups.

CONCLUSIONS

Study Director's Comments: The results of the spot test demonstrated no mutagenicity of the test sample with rat, with mouse, or without microsomal activation, at a concentration of 25 mg of sample per disk using strains TA98, TA100, TA1535, and TA1537. The results of the Salmonella plate incorporation assay demonstrated no mutagenicity of the test sample to any of the four tester strains (TA98, TA100, TA1535, or TA1537), either with or without a rat microsomal activation system, at concentrations ≤ 10 mg of sample per plate. Toxicity assays with strain TA100 indicated no toxicity of the sample to the tester strain at a concentration ≤ 10 mg of sample per plate.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: The study is similar in method/design to OECD guideline 471. Only 4 strains run versus 5 called for by OECD guideline.

REFERENCES (Free text):

Gridley, J. and Ross, W.D.; Salmonella Mutagenicity Assay of CP4789, DA-80-020 (Sodium Cyanurate/Cyanuric Acid); Unpublished Study Conducted by Monsanto Research Corporation, St. Louis, MO; Report No. MRC-DA-969, Study No. Study No. 80-1-14-220-00A; May 28, 1980.

Hammond, Bruce G, Barbee, Steven J., Wheeler, Allan G, and Cascieri, Tito, "Absence of Mutagenic Activity for Monosodium Cyanurate," Fundamental and Applied Toxicology, Vol. 5, pp. 655-664 (1985).

16a

Repeat Dose Toxicity

TEST SUBSTANCE

• Identity: Monosodium cyanurate (sodium cyanurate monohydrate); CAS No. 2624-17-1
• Remarks field for Test Substance:
- Purity: 99.5%
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 408 "Repeated Dose 90-Day Oral Toxicity Study in Rodents"
• Test type: Repeat Dose
• GLP (Y/N): Not identified in Study Report
• Year (study performed): 1981
• Species: Mice
• Strain: $B_6C_3F_1$ (Charles River)
• Route of administration: Oral, drinking water
• Duration of test: 13 weeks
• Doses/concentration levels: 0, 896, 1792, 5375 ppm
• Sex: Male/female
• Exposure period: 13 weeks
• Frequency of treatment: *ad libitum*
• Control group and treatment: Two; vehicle (tap water) and sodium (sodium hippurate)
• Post exposure observation period: None
• Statistical methods: The growth rates, total food consumption, water consumption, clinical pathology (except leukocyte differentials, red blood cell morphology, and select urine data and organ weight data of the control group (Group 1) were compared statistically to the data of the treated groups of the same sex by Bartlett's test for homogeneity of variance (Bartlett, 1937). This analysis was followed by a one-way classification analysis of variance (ANOVA) (Snedecor and Cochran, 1957) if the variances proved to be homogeneous. If the variances proved to be heterogeneous a $\log_{10}$ transformation was performed, which was followed by Bartlett's test. If the $\log_{10}$ transformation was ineffective in removing variance heterogeneity, a $\log_8$ transformation of the original data was performed which was followed by Bartlett's test. If homogeneity could not be achieved by transformation, ANOVA of the nontransformed data was completed. If ANOVA of homogeneous data was significant, Scheffe's (1953) multiple pairwise comparison procedure was used to compare the group mean values. If ANOVA of heterogeneous data was significant, Games and Howell's (1976) multiple pair-wise comparison procedure was used to compare the group mean values. All analyses were evaluated at the 5.0% probability (one-tailed) level.
• Remarks field for Test Conditions:
- Age at study initiation: 6 weeks (Males: 11.7-22.1 gms; Females: 13.11-18.5 gms)
- No. of animals per sex per dose: 25
- Vehicle: Water
- Clinical observations: Animals were inspected 3 times daily for mortality and moribundity. Body weights, food consumption, and clinical observations were made weekly. Water consumption was measured twice weekly. Hematology analyses were performed on 10 animals/sex/group after 13 weeks of treatment. Urinalysis was performed on 10 animals/sex/group after 13 weeks of treatment.
- Organs examined at necropsy (macroscopic and microscopic):
6 week sacrifice - After six weeks of treatment, five animals/sex/group were sacrificed. The kidneys were weighed. A wide range of tissues and organs were preserved in 10% neutral buffered formalin.
Specimens of kidney, urinary bladder, ureter, and any gross lesions were subjected to microscopic examination from the control(s) and high-dose (5375 ppm) animals sacrificed after six weeks of treatment.
Terminal Sacrifice (13 weeks) - All remaining animals were sacrificed at 13 weeks. The following organs were weighed: liver, heart, kidneys, testes and epididymis, ovaries, brain. Specimens of kidneys, urinary bladder, ureter, liver, heart, brain, testes or ovaries,

lung and any gross lesions were examined microscopically from all animals in the control(s) and high-dose (5375 ppm) groups sacrificed after thirteen weeks of treatment. The kidneys, urinary bladder, ureter, and any gross lesions obtained from the terminally sacrificed 896 and 1792 ppm dose level animals were also examined microscopically.

RESULTS

• Actual dose received by dose level by sex: Estimates of the mean daily consumption of cyanurate (mg/kg body weight/day) for the thirteen-week period are presented below:

Group:		Males			Females	
	896 ppm	1792 ppm	5375 ppm	896 ppm	1792 ppm	5375 ppm
Mean	252.0	522.4	1994.2	298.0	609.6	2200.7
S.D.	26.3	45.5	116.4	31.9	53.5	186.0
N	7	7	7	7	7	7

Compound consumption (mg/kg) was higher in females at all ages in all groups due to the normally greater water intake (ml/kg) of the females. Compound consumption was highest at the beginning of the study and decreased throughout the study due to the normal decrease of water consumption with growth in mice.

Estimates of the mean daily sodium consumption (mg/kg/body weight/day) for the thirteen-week period are presented below.

	Males				Females			
Group:	896 ppm[a]	1792 ppm[a]	5375 ppm[a]	7769 ppm	896 ppm[a]	1792 ppm[a]	5375 ppm[a]	7769 ppm
Mean	32.3	66.9	255.3	260.2	38.2	78.0	281.7	302.7
S.D.	3.4	5.8	14.9	22.8	4.1	6.8	23.8	34.6
N	7	7	7	13	7	7	7	13

a = based on the amount of sodium present in monosodium cyanurate (12.8%, as provided by sponsor)

• Toxic response/effects by dose level: No unusual or compound-related clinical signs were reported. Alopecia was noted in all groups at approximately equal frequencies.
• Statistical results, as appropriate: N/A
• Remarks field for Results:
- Body weight: Analysis of growth through Weeks 6 and 13 did not reveal any significant differences. No apparent trends were noted in the weekly mean data. Means of the treated groups of either sex differed by one gram or less from the respective control group.

Males	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
0	Mean (g) Std. Dev	17.8 1.49	18.2 1.52	18.6 1.94	18.3 1.66	17.7 2.26
6		22.8 1.42	22.4 1.52	23.2 2.11	22.8 2.02	22.1 2.53
13		25.2 1.71	25.2 1.64	25.4 2.42	25.6 2.26	24.4 2.88

Females	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
0	Mean (g) Std. Dev	15.9 1.11	15.9 1.22	16.1 0.73	15.8 0.93	15.8 1.07
6		21.7 1.63	21.5 1.30	22.1 1.15	22.3 1.25	22.5 1.04
13		24.1 1.31	23.5 1.35	23.9 1.78	23.4 1.11	23.8 0.98

- Food Consumption: Analysis of total food consumption through Weeks 6 and 13 did not reveal any significant differences between control and treated groups.

Males	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
1-6	Mean (g/wk) Std. Dev	248.7 28.3	240.6 22.3	247.9 25.8	247.7 23.9	256.0 21.2
1-13		553.7 53.5	534.7 45.3	541.0 60.5	557.9 55.9	585.1 36.2

Females	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
1-6	Mean (g/wk) Std. Dev	259.4 20.2	257.2 24.5	259.0 19.2	263.8 23.0	260.6 19.5
1-13		594.0 43.7	585.2 57.5	590.8 52.4	591.7 54.2	585.0 46.9 to

- Water Consumption: Consistently higher than control mean values were noted in the 5375 ppm cyanurate animals of both sex. In the males, this difference was statistically significant at all weeks except Weeks 1, 2, and 8. In the females, this difference was not significant at Weeks 1 through 5 and 10 and 12. The elevated water consumption values are considered treatment related.

Males	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
1	Mean (g) Std. Dev	48.1 16.64	43.9 12.31	44.9 7.55	55.6 13.51	49.4 7.37
6		42.9 7.10	43.9 8.36	46.3 8.49	56.7* 8.17	44.7 7.03
13		38.4 11.37	37.7 6.79	42.4 13.91	56.7* 9.73	44.3 9.84

* $p<0.05$

Females	Dose Level	0 ppm	896 ppm	1792 ppm	5375 ppm	7769 ppm sodium
Weeks on study						
1	Mean (g) Std. Dev	47.3 18.25	49.1 12.62	48.6 5.99	57.9 10.10	53.7 7.46
6		49.7 9.89	48.4 10.61	44.9 8.73	61.4* 6.07	54.4 14.09
13		42.1 7.20	41.9 6.76	44.41 13.98	61.1* 23.28	47.3 11.69

* $p<0.05$

- Description, severity, time of onset and duration of clinical signs: No unusual or compound-related clinical signs were reported.
- Hematological findings incidence and severity: No compound-related hematological abnormalities were reported. Statistical evaluation revealed lower mean platelet counts in males at the 896 and 5375 ppm cyanurate dose levels. This finding was considered incidental by the study directors as the mean values were well within the reference range for this species at the laboratory. No other differences were noted.
- Clinical biochemistry findings incidence and severity:

Urinalysis: Statistical evaluation revealed incidental decreases in the mean chloride level in the 896 ppm cyanurate males and the

urine volume of the Sodium control male, and an increase in urobilinogen in the 5375 ppm cyanurate females. No other apparent differences or patterns were noted.
- Mortality and time to death: A single male in the 5375 ppm cyanurate group died accidentally at week 11.
- Gross pathology incidence and severity: No compound related abnormalities were reported at necropsy. At the week 13 sacrifice, calculi or granular material were noted in the urinary bladder of one male in the 896 ppm group and 2 males in the 5375 ppm cyanurate groups.
- Organ weight changes: No compound-related differences in week 6 kidney weights were reported.
Dose-related increases in absolute and relative ovarian weights were observed in the week 13 data. The increase was statistically significant for the high-dose (5375 ppm) cyanurate and positive control (7769 ppm sodium). The response was attributed by the study director and EPA reviewers as most probably related to the sodium intake. Analysis of all other absolute and relative organ weights found no test compound related effect.

Group (ppm)		Body Wt (g)	Ovaries Wt, Absolute (g)	Ovaries Wt, Relative (%)
0	Mean Std. Dev.	20.07 1.220	.0148 .08319	.0739 .01646
896		20.15 1.292	.0182 .00337	.0907 .01663
1792		20.01 1.407	.0182 .0039	.0910 .01899
5375		19.80 1.481	.0196* .00443	.0990* .02138
7769 Sodium control		20.25 .888	.0199* .00444	.0987* .02284

* $p < 0.05$

- Histopathology incidence and severity: No treatment-related abnormalities were seen in the tissues examined microscopically from animals sacrificed after 6 weeks of exposure. A variety of spontaneous disease lesions and incidental findings were noted as follows: in the kidneys, perivascular accumulations of mononuclear cells were noted in two control mice and in one Sodium control 5 male. A single dilated tubule was noted in the kidney of one control female and minimal focal nephropathy was present in one Sodium control male. Urinary bladder sections were not remarkable with the exception of focal submucosal mononuclear infiltrate in one Sodium control female. Sections of ureters from all mice were histologically normal. Stomach sections were examined from a single control female and two females in the 5375 ppm cyanurate and Sodium control groups with a finding of focal ulceration in the two 5375 ppm females. Spleen sections revealed congestion in two 5375 ppm cyanurate mice and in one Sodium control mouse with constriction as a developmental variation in a control male. Liver sections revealed foci of mononuclear cells in a control male and subcapsular necrosis in a Sodium control male.

Microscopic examination of tissues from animals sacrificed after 13 weeks of exposure found histological alterations in the bladder lining in two male mice from the 5375 ppm cyanurate group. Calculi were present in the bladder of these animals. These changes consisted of hyperplasia of the transitional epithelium and congestion or hemorrhage and were associated with the presence of calculi in the bladder of the mice. Sections of urinary bladder from all other 5375 ppm group mice were within normal histologic limits. No other treatment-related abnormalities were observed.

A variety of spontaneous disease lesions and incidental findings were noted as follows: In the lungs, early lesions of murine pneumonia consisted of perivascular mononuclear proliferation and peribronchial lymphoid proliferation as well as the presence of alveolar macrophages and occasional foci of pneumonitis. Heart sections were unremarkable with the exception of focal nonsuppurative myocarditis in a control male. Foci of mononuclear cells were noted in liver sections from occasional control and treated mice and focal hepatic necrosis was noted in a control male and in five mice in the high-dose monosodium cyanurate group as a spontaneous disease lesion. Kidney sections revealed perivascular mononuclear infiltrate in occasional mice of all groups in addition to focal nephropathy. These lesions occurred without relationship to treatment and were of essentially comparable incidence and severity in the control and treated group. Focal hyperplasia of renal tubule epithelium was noted in single mice in the control, 896, 5375 and Sodium control groups.

With the exception of the urinary bladder changes associated with the presence of calculi in the bladders of the two 5375 ppm group males, the only other findings observed were focal submucosal mononuclear infiltrate which occurred in mice of all groups. A variety of other findings were observed infrequently and without association to treatment of which the most common were hydrometra and cystic endometrial hyperplasia in uterine sections.

CONCLUSIONS

Study Director's Comments: "Consistently higher water consumption was noted in the 5375 ppm cyanurate dose group males and females. This difference was significant for all weeks except Weeks 1, 2, and 8 for the males and Weeks 1, 2, 3, 4, 5, 10, and 12 for the females. No other compound-related effects were noted during the in-life phase.

... administration of monosodium cyanurate at a level of 5375 ppm and the sodium control at 7769 ppm in the drinking water for six weeks failed to cause compound-related histomorphologic alterations in the tissues examined. A variety of spontaneous disease lesions and incidental findings were noted apparently without relationship to treatment."

…administration of monosodium cyanurate at a level of 5375 ppm and the sodium control at 7769 ppm in the drinking water for thirteen weeks failed to cause compound-related histomorphologic alterations in the tissues examined with the exception of changes in the urinary bladder resulting from the presence of calculi in two male mice in the high-dose monosodium cyanurate group. A variety of spontaneous disease lesions and incidental findings were noted with essentially comparable incidence and severity in the control and treated groups and without relationship to treatment."

EPA Data Evaluation Report: The compound was administered in the drinking water where the highest dose possible was limited to a water concentration of 5375 ppm by solubility. This produced mean high-dose consumption rates of 1994.2 mg/kg/day (males) and 2200.3 mg/kg/day (females). The only compound-related effect of this dose was urinary bladder calculi in two males accompanied by irritation of the lining of the bladder. Since human exposure to cyanurate is in the water of swimming pools, the high dose is sufficient for the purposes of the Study.

DATA QUALITY

• Reliability: Klimisch Code 3c (Not reliable, does not meet important criteria of today's standard methods) does not meet OECD Guideline 408, ophthalmological exam, sensory reactivity observation, no clotting time on hematology, no clinical biochemistry, limited histopathology)
• Remarks field for Data Reliability: The EPA Data Evaluation Record classifies this study as "Guideline". It goes on to state: "Although there is no requirement for a 90-day oral study in the mouse, the study as performed satisfies the technical standards for a 90-day oral dosing study in rodents."

REFERENCES (Free text):

"Thirteen-Week Toxicity Study in Mice, Monosodium Cyanurate", Hazleton Laboratories America, #2169-101, Feb 7, 1986, Accession Numbers 2169-100, May 11, 1982.

EPA Data Evaluation Record, August 1982, MRID 124782.

16b

Repeat Dose Toxicity

TEST SUBSTANCE

• Identity: Sodium cyanurate monohydrate (s-triazinetriol, monosodium salt); CAS No. 2624-17-1
• Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 408 "Repeated Dose 90-Day Oral Toxicity Study in Rodents"
• Test type: Repeat Dose
• GLP (Y/N): Yes
• Year (study performed): 1980
• Species: Rat
• Strain: CD® (Charles River)
• Route of administration: Oral, drinking water
• Duration of test: 13 weeks
• Doses/concentration levels: 0, 896, 1792, 5375* ppm (*limit of solubility in water)
• Sex: Male/female
• Exposure period: 13 weeks
• Frequency of treatment: *ad libitum*
• Control group and treatment: Two; vehicle (tap water) and sodium (sodium hippurate at 7812 ppm), 40 males and females
• Post exposure observation period: None
• Statistical methods: All statistical analyses compared the treatment groups with the control groups, by sex.
Body weights (week 13), hematological, biochemical and urinalysis parameters (weeks 6 and 13 of the main study, and 2, 4, 6, 8 and the 10 week interim sacrifice) and absolute and relative organ weights (2, 4, 6, 8, 10 and terminal sacrifices) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.
• Remarks field for Test Conditions:
- Age at study initiation: 21 days (Males 82-106 gms; Females 78-96 gms)
- No. of animals per sex per dose:

Group	(ppm)	Test article	Male	Female
I	0	(negative control)	40	40
II	896	s—triazinetriol monosodium salt	24	24
III	1792	"	24	24
IV	5375	"	40	40
V	7812	sodium hippurate (sodium control)	40	40

- Vehicle: Water
- Clinical observations: Rats were observed twice daily for signs of toxicity, morbidity and mortality. Detailed observations were conducted weekly. Individual body weights and food consumption were recorded weekly.
Water consumption was measured daily. An extensive list of hematology, clinical chemistry and urine analyses were performed as follows: 10 rats/sex during the pretest period; 10 rats/sex/group at 6 and 13 weeks of study; and, 4 rats/sex from the 5375 ppm cyanurate, negative and sodium control groups at weeks 2, 4, 6, 8 and 10 of the study. Animals sacrificed and necropsied.
- Organs examined at necropsy (macroscopic and microscopic):
In addition to the terminal sacrifice at 13 weeks, interim sacrifices were conducted at 2, 4, 6, 8 and 10 weeks of the study.
All animals sacrificed at scheduled necropsies were subjected to detailed gross necropsy. For each animal, a wide range of tissues and organs were preserved. At the interim sacrifices the kidneys were weighed. At the terminal sacrifice the liver, kidneys, heart, testes, brain with stem and ovaries were weighed. Microscopic examination of hematoxylin and eosin stained paraffin sections of the

kidneys, ureters, and urinary bladder were conducted in the control, sodium control and high-dose rats sacrificed at the 2, 4, 6, 8, and 10-week interim sacrifices, and rats found dead during the course of the study. Sections were also prepared and examined from an extensive list of tissues of both control groups and the high-dose group rats sacrificed at the end of the study. In addition, sections were prepared and examined from the heart, liver, urinary bladder, kidneys, ureters and all gross lesions of terminal sacrifice rats in both mid-dose groups.

RESULTS

• NOAEL (NOEL): >5375 – 5375 ppm
EPA Data Evaluation Record:
In the males the NOEL for increased water consumption ranges from 357 to 145 mg/kg, middle dose, (week 1 to week 13). In the females this effect was not demonstrated giving a NOEL ranging from 1204 to 763 mg/kg, high dose, (week 1 to week 13). The actual doses at which the females did not show this effect are up to 1.5 times higher than the doses at which the males did show the effect.
In the males the NOEL for bladder hyperplasia ranges from 165 to 72 mg/kg, low dose, (week 1 to 13). In the females this effect was not demonstrated giving a NOEL ranging from 1204 to 763 mg/kg, high dose (week 1 to week 13). As noted above the actual dose to females was up to 1.5 times the dose to males at the same ppm cyanurate in the drinking water.
• LOAEL (LOEL): Not identified by the study directors.
• Actual dose received by dose level by sex:

EPA Data Evaluation Record

	Mean Dose Males (mg/kg)				
Week of Study	0 ppm (Control)	896 ppm	1792 ppm	5375 ppm	7812 ppm (Na Control)
1	0	165	357	1113	1572
2	0	160	330	1093	1476
3	0	130	271	887	1203
4	0	116	247	806	1070
5	0	96	208	817	898
6	0	103	215	693	914
7	0	90	192	629	805
8	0	82	181	591	758
9	0	82	174	575	719
10	0	72	163	553	612
11	0	70	153	500	625
12	0	69	147	484	633
13	0	72	145	495	625

		Mean Dose Females (mg/kg)			
Week of Study	0 ppm (Control)	896 ppm	1792 ppm	5375 ppm	7812 ppm (Na Control)
1	0	184	378	1204	1672
2	0	177	358	1215	1664
3	0	148	312	994	1367
4	0	139	281	908	1289
5	0	121	290	806	1172
5	0	130	272	860	1266
7	0	125	249	849	1219
8	0	123	247	812	1172
9	0	113	231	774	1109
10	0	110	215	731	977
11	0	103	206	704	969
12	0	105	204	693	922
13	0	108	208	763	927

• Remarks field for Results:
- Body weight: No changes considered compound related occurred in group mean body weights at 13 weeks of study. There were, however, statistically significant increases in mean body weight for the 896 ppm females and l792 ppm males at 13 weeks; these changes were of doubtful biological significance. They were not considered compound related by either the study directors or the EPA Data Evaluation Record.

Group	Dosage Level (ppm)	Test Article	Mean Body Weights, g (% Difference from the Control)	
			Male	Female
I	0 (Control)	--	455	255
II	896	s-triazinetriol, monosodium salt	457 (+0.4)	270 (+5.9)
III	1792	“	489 (+7.5)	257 (+0.8)
IV	5375	“	453 (-0.4)	253 (-0.8)
V	7812	sodium hippurate	446 (-2.0)	252 (-1.2)

- Food Consumption: No changes considered compound related occurred in group mean food consumption values through 12 weeks of study. (Due to technical error, mean food consumption values were not calculated for week 13.) The occasional increases in food consumption values somewhat paralleled those increases noted in group mean body weights.

Group	Dosage Level (ppm)	Test Article	Mean food Consumption [g/rat/day] (% difference from Control)	
			Male	Female
I	0 (Control)	--	25.3	18.5
II	896	s-triazinetriol, monosodium salt	25.1 (-0.8)	19.2 (+3.8)
III	1792	"	26.4 (+4.3)	18.4 (-0.5)
IV	5375	"	25.5 (+0.8)	18.5 (0)
V	7812	sodium hippurate	25.3 (0)	18.7 (+1.1)

- Water Consumption: No trends in group mean water consumption values were considered compound related through 13 weeks of study. Although mean water consumption increases were noted for the 1792, and 5375 ppm males and decreases for the 896 and 1792 ppm females through 13 weeks, these changes were of doubtful biological significance.

Group	Dosage Level (ppm)	Test Article	Mean Water Consumption [g/rat/day] (% difference from Control)	
			Male	Female
I	0 (Control)	--	37.4	32.7
II	896	s-triazinetriol, monosodium salt	36.1 (-3.5)	31.8 (-2.8)
III	1792	“	40.3 (7.8)	30.4 (-7.0)
IV	5375	“	42.3 (+13.1)	33.7 (+3.1)
V	7812	sodium hippurate	36.8 (-1.6)	36.3 (+11.0)

- Hematological findings: In the main study and in the interim sacrifice study there were scattered statistically significant differences between the non-treated control, the sodium hippurate control and the test compound treated mean values. However, these differences were well within the normal range, showed no discernible trends and were not considered to be test compound related by either the study directors or the EPA Data Evaluation Record.
- Clinical biochemistry findings: In the main study several trends in chemistry test results were identified. Total protein and globulin levels appeared to decrease with increasing sodium intake. There was not a significant difference between total protein and globulin levels for 5375 ppm treated males and sodium control males. However, there was a significant difference between 5375 ppm treated females and sodium control females at the 6 week intervals but not the 13 week interval. The contribution of the test compound to protein alterations is therefore equivocal. Similarly, calcium was decreased with sodium intake in time for both males and females. No consistent alterations were seen in calcium levels at the 6 week interval. However, at the 13 week interval there were statistically

significant dose related differences between the compound treated test groups and the untreated controls, but not between the 5375 ppm group and the sodium control group. The origin of the decreased calcium in association with sodium treatment is uncertain. Decreased calcium cannot be explained by the decreased total protein since calcium is bound to albumin which remains unchanged throughout the study; nor does this decreased calcium appear to be test compound related. Small but statistically significant decreases were seen in chloride levels in males in all test compound groups and in females in the 5375 group. This change appears to be compound related.
In addition to the trends described above, occasional statistically significant alterations were seen in many test group means but because of their random nature were not considered test compound related. It is to be emphasized that all of the changes described above are of a minor degree and that all test group means fall within acceptable normal limits. The biological significance of any of the changes is therefore equivocal. Indeed, the trends in the main study were not seen in the interim sacrifice groups where only random changes were identified.
- Urinalysis Findings: No apparently compound-related changes were seen in the urinalysis data, although scattered statistically significant alterations were identified with numerous tests.
- Mortality and time to death: No trend in mortality suggestive of a compound-related effect occurred through 13 weeks of study.

Group	Dosage Level (ppm)	Test Article	Number Surviving/Number Initiated*	
			Male	Female
I	0 (Control)	--	20/ 20	**19/20
II	896	s-triazinetriol, monosodium salt	24/24	24/24
III	1792	“	24/24	24/24
IV	5375	“	20/20	20/20
V	7812	sodium hippurate	20/20	**19/20

*Less interim sacrifice of 4 rats/sex in groups I, IV and V conducted at weeks 2, 4, 6, 8 and 10.
** Died during blood collection

- Gross pathology findings: There were no macroscopic changes which could be attributed to the compound.
- Organ weight changes: Statistically significant ($p < 0.05$) increases in relative kidney weight of males in the 5375 ppmgroup occurred at the 4 and 10 week interim sacrifice. Statistically significant variation occurred in some absolute and/or relative weights of liver, kidneys, testes, heart and brain at several dose levels and in either sex. There was no dose relationship or statistically significant variations in females of the high dose (5375 ppm) group when compared to either control group (0 ppm or 7812 ppm Sodium Control). There was a statistically significant decrease ($p < 0.05$) in relative weight of testes and heart for the males of the high-dose group (5375 ppm) when compared to the sodium control (7812 ppm sodium). Because of the lack of a dose response, the variations were not related to the compound.
- Histopathology: Hyperplasia of the urinary bladder epithelium occurred in four of 20 male rats at the 5375 ppm dosage level and in one of 24 male rats at the 1792 ppm dosage level at terminal sacrifice. This lesion was not present in the other terminal sacrifice groups. The hyperplasia was “very slight” to “slight” in severity and was found in males but not females.
Hyperplasia of the urinary bladder epithelium was present in one of four males at the 5375 ppm dosage level at the 6 week interim sacrifice, one of four males in the same dose at the 8 week interim sacrifice and in two of four males in the same dose group at the 10 week interim sacrifice. One of four females had hyperplasia of the bladder epithelium in the sodium control group at the 8 week interim sacrifice. No hyperplastic lesion occurred in the 0 ppm control rats at any interim sacrifice period. All of the hyperplastic lesions observed in interim sacrifice were “very slight” to “slight” in severity.
Based on the incidence pattern found in terminal sacrifice and interim sacrifice rats, it is concluded that hyperplasia of the urinary bladder epithelium was compound related in the male but not the female rats in this study.
The other microscopic changes were non-specific, spontaneous lesions unrelated to the compound.

EPA Data Evaluation Record: Regarding the possibility that the bladder hyperplasia might be caused by calculi.
“The gross necropsy report on the male at 6 weeks leads one to question whether this is part of the compound response or a traumatically damaged bladder.”

CONCLUSIONS

Study Director’s Comments: No clinical signs considered compound related occurred through 13 weeks of study. Corneal opacity was noted frequently for control and treated rats. No changes considered compound related occurred in survival, body weights, food or

water consumption throughout the study.
Although there were scattered statistically significant changes in some biochemical values, these were of a random nature and therefore were of equivocal biological significance and not considered related to the test article.
There were no macroscopic changes which could be attributed to the compound. There were no compound related variations in organ weights. Histologically, hyperplasia of the urinary bladder epithelium resulted from compound treatment in the males but not the females. This lesion was present at the 6, 8, and 10 week interim sacrifice, and at the terminal sacrifice. Based on the incidence pattern found in terminal sacrifice and interim sacrifice rats, it is concluded d that hyperplasia of the urinary bladder epithelium was compound related in the male but not the female rats in this study.

EPA Data Evaluation Report: "…no major compound related effects were demonstrated. Compound related effects were minor in nature, scattered and did not appear to be part of a pattern. There is no evidence of diuretic activity.
There is no evidence of a diuretic effect in this study. Urine concentrating ability and urine composition show no compound related abnormalities. Plasma electrolytes, creatinine, BUN and blood osmolality show no compound related abnormalities, These parameters would be expected to be effected by abnormal kidney function such as diuresis and together are a sensitive indicator of kidney function."

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study) (OECD Guideline 408)
• Remarks field for Data Reliability: No ophthalmological examination, no outside home cage weekly observations, no sensory reactivity to stimuli/grip strength/motor activity, organ weights not done on spontaneous, limited histopathology)
The EPA Data Evaluation Record classifies this study as "Guideline."

REFERENCES (Free text):

"S-Triazinetriol, Monosodium Salt, 13-Week Toxicity Study in Rats", International Research and Development Corporation, Report Number #167-151, March 17, 1981.

EPA Data Evaluation Record, August 1982, MRID 63477.

16c

Repeat Dose Toxicity

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- 77.5% cyanuric acid
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 451 "Carcinogenicity Studies"
• Test type: Carcinogenicity
• GLP (Y/N): Not identified in Study Report
• Year (study performed): 1982-1984
• Species: Mice
• Strain: $B_6C_3F_1$
• Route of administration: Oral, drinking water
• Duration of test: 104 weeks
• Doses/concentration levels: 0, 100, 400, 1200, 5375 ppm (5375 ppm was the maximum solubility for monosodium cyanurate in water)
• Sex: Male/female
• Exposure period: 104 weeks
• Frequency of treatment: *ad libitum*
• Control group and treatment: Two; vehicle (tap water) and sodium (sodium hippurate)
• Post exposure observation period: None
• Statistical methods:
Cumulative survival data through Week 104 were analyzed using the National Cancer Institute Package (Thomas, Breslow, and Gart, 1977). Groupwise comparisons were based on an analysis of all groups, using the highest concentration of sodium hippurate that occurred on study as the dose level for Group 6. Trend analyses were evaluated using Groups 1 through 5 only.
Food consumption, water consumption, body weight, hematology (except leukocyte differentials and morphology), serum chemistry, urine chemistry, and organ weight data were evaluated statistically. Food consumption was totaled for the intervals, initiation through Week 13, initiation through Week 26 and initiation through Week 52, and then analyzed; whereas, water consumption was analyzed separately throughout the study. Absolute body weights at initiation and Weeks 13, 26, and 52 were analyzed. In addition, growth rates were analyzed in the same manner at Weeks 13, 26 and 52. Absolute body weights (prior to sacrifice), absolute organ weights, organ/body weight ratios, and organ/brain weight ratios for the Weeks 27, 53, and 79 interim sacrifices and the terminal sacrifice were analyzed.
Analyses of the aforementioned data were performed in the following order: Levene's test for homogeneity of variances (Levene, 1960; Draper and Hunter, 1969) was performed, and if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA) (Winer, 1971). If the variances proved to be heterogeneous, a series of transformations was performed until variance homogeneity was achieved. If one of the transformations was effective in achieving variance homogeneity, ANOVA of the transformed data was performed. If ANOVA of untransformed or transformed data was significant, the Games and Howell modification of the Tukey-Kramer honestly significant difference test (Games and Howell, 1976) was used for group mean comparisons. If ANOVA was not significant, the analysis was complete. If none of the transformations were effective in achieving variance homogeneity, the Terpstra-Jonckheere (Terpstra, 1952; Jonckheere, 1954) nonparametric test for trend and Kruskal-Wallis nonparametric one-way ANOVA and pairwise group comparisons (Kruskal and Wallis, 1952) were performed.
Levene's test, ANOVA, the Terpstra-Jonckheere test, and the Kruskal-Wallis ANOVA were evaluated at the 5.0% one-tailed probability level. Group comparisons by the Games and Howell test or the Kruskal-Wallis test were evaluated at the 5.0% and 1.0% two-tailed probability levels.
• Remarks field for Test Conditions:
- Test Subject Age at study initiation: 6 weeks

- No. of animals per sex per dose:

Group	Concentration (ppm)	Number of Mice	
		Males	Females
1	0	100	100
2	100	80	80
3	400	100	100
4	1200	100	100
5	5375	100	100
6 (Sodium Control)	8005-10281	80	80

- Vehicle: Water
- Clinical observations: Animals were observed for mortality and moribundity three times daily. Detailed observations for clinical signs and tissue masses were performed weekly. Individual body weights and food consumption were measured weekly from initiation through Week 14, and biweekly thereafter. Individual water consumption was measured twice each week. Hematologic and serum chemistry analyses were performed on 10 animals/sex/group before treatment, and at 52 and 104 weeks. Urinalysis was performed on 10 mice/sex prior to initiation and on 10 mice/sex/ group after Weeks 26, 52, 78, and 104.
- Organs examined at necropsy (macroscopic and microscopic): All animals that were sacrificed as scheduled, or that were found dead or died in extremis were necropsied. All necropsies included examination of the following: the external surface, all orifices, cranial cavity, carcass, external surface of the brain and spinal cord, the nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs. At necropsy, the following organs were weighed: brain, heart, liver, kidneys, testes (with epididymis) and ovaries. The urinary bladder was opened and examined for each animal. If there was any indication that calculi were present (e.g., distension or blood tinged urine) the urinary bladder was opened in a petri dish to prevent possible loss of calculi. Any calculi were placed in a labeled vial and frozen. All preserved tissues from animals that were found dead or sacrificed in extremis as well as all control and high-dose animals at terminal sacrifice were examined microscopically. For animals surviving to study termination, coronal sections of the head were examined microscopically from 10 animals per sex from each group. The kidneys, ureters, urinary bladder, and gross lesions from 10 mice per sex from control and high-dose groups at interim sacrifices were also examined microscopically.

RESULTS

• NOEL: 5387 ppm from EPA Data Evaluation Report
• LOEL: Not identified
• Actual dose received by dose level by sex, if known:

	Group	Compound Consumption (ppm)	
		Males	Females
2		23.69	25.90
3		96.58	100.14
4		306.90	315.42
5		1523.26	1582.20
6	(sodium control)	2093.09	2218.79

• Toxic response/effects by dose level: No treatment-related effects on survival, clinical pathology (except urine sodium), organ weights, gross pathology, and histopathology were observed. Apparent treatment-related effects on clinical signs (swollen/enlarged abdomens), body weights, food consumption, and urine sodium were generally observed in both sexes treated with monosodium cyanurate at higher levels and in the sodium control group. The significance of these responses, however, is unclear in terms of monosodium cyanurate treatment. These effects may well be the result of treatment with high levels of sodium.
• Statistical results, as appropriate: N/A
• Remarks field for Results:
- Body weight: Statistical analyses revealed significantly lower mean absolute body weight values at Weeks 13 and 26 in the Group 5 females compared to the Group 1 control females. Mean growth rates for the interval, initiation through Week 13 were significantly lower in the Groups 3, 4, 5, and 6 females, and in the Group 5 females for the interval, initiation through Week 26. At Week 52, no

statistically significant differences were noted in the body weights or growth rates of control and cyanurate-treated females. No significant differences were noted in the male body weight data.
- Food Consumption: Statistically significantly lower mean total food consumption values were noted in the Group 4 males and females at Weeks 13 and 26 as well as at Week 52 for the males. Total food consumption was significantly lower at weeks 26 and 52 in the males but was due to the influence of the decreased food consumption noted during the first 13 weeks. During most weeks throughout the study, food consumption was higher for the Group 5 males than for the Group 6 sodium control males. For the females, however, food consumption was generally lower for Group 5 than for Group 6 for the first 13 weeks and was comparable for those two groups after Week 13. In addition, the mean total food consumption value for the first 13 weeks in the Group 6 males was significantly lower than the Group 1 control value.
- Water Consumption: Group 5 mean water consumption values in the males were moderately to significantly higher than the Group 1 control males. In addition, the Group 5 mean value was higher than the Group 6 mean values for both sexes. These notably increased values would tend to indicate that the increased water consumption noted in Group 5 may bear some relationship to treatment. Also in support of this, mean water consumption values in the Group 4 males were generally higher (occasionally significantly) than in the Group 1 control after Week 10. Mean water consumption values in the other groups (Groups 2 and 3 males and Groups 2, 3, and 4 females) were generally comparable to the Group 1 control males and females. Although not tested for statistical significance because of variance homogeneity, the mean water consumption values generally appeared indicative of a positive trend for Groups 1-5 throughout the study; this was noted more often for males than for females.
- Description, severity, time of onset and duration of clinical signs: Increased incidences of swollen/enlarged abdomens were noted for the Groups 4, 5, and/or 6 males (compared to the Group 1 control males) beginning Week 15 and continuing throughout the study (with few exceptions). In addition, the incidences in the Group 5 males continued to be higher (with few exceptions) than in the Group 6 males. In the Groups 2 and 3 males, the incidences of swollen/enlarged abdomens were generally similar to the Group 1 control group. In the females, swollen/enlarged abdomens were observed less frequently than in the males, but after Week 27, the incidences in the Groups 4, 5, and 6 females were slightly higher during several weeks than in the Group 1 control females.
In general, a slightly increased number of occurrences of rough hair-coat were noted in the Groups 4, 5, and 6 females compared to the control females (Group 1). Sporadic findings of tissue masses (including wartlike lesions) were observed in all groups of females except Group 4. The incidence of these findings was low and did not exceed 3.3% of the number of surviving females of the groups at any given interval. Tissue masses were not observed in any of the male groups. Incidences of other clinical signs were similar in control and treated groups of both sexes.
- Hematological findings incidence and severity: Evaluation of hematology data revealed no statistically significant differences between the control and treated groups and no indications of compound-related effects.
- Clinical biochemistry findings incidence and severity: Statistical analysis of serum chemistry data revealed a significantly higher BUN level for Group 6 females when compared to the Group 1 females at termination. This effect was not considered treatment related since BUN values for all female groups at Week 53 and for all male groups at both Weeks 53 and 105 were comparable.
At all collection intervals, mean urine sodium concentrations and excretion values were noted to be slightly to significantly higher in the Groups 5 and 6 females when compared to the Group 1 control group. At Weeks 27, 79, and 105, the mean sodium values for the Group 6 females were generally higher than for the Group 5 females. At Week 53, the mean sodium values for Groups 5 and 6 females were similar. Statistically significant increases were also noted in the mean sodium concentrations and excretion values of the Groups 5 and 6 males at Week 79.
- Mortality and time to death: Survival was similar in all groups throughout the study and no statistically significant differences between groups or trends were revealed at termination.
- Gross pathology incidence and severity: No distinct compound-related trends were observed in the gross pathology incidences.
- Organ weight changes: Slightly to significantly lower mean absolute ovary weights, ovary/body weight ratios, and ovary/brain weight ratios were observed in Groups 2-6 when compared to the Group 1 control for females sacrificed at Week 27 as well as Week 53. When the ovary weight of one Group 1 female was excluded from analyses (because the weight was approximately 10 times the average), mean ovary weights and ratios were similar in Groups 1 and 2, but means were still slightly to significantly lower in Groups 3-6 compared to Group 1 at Week 53. At the 27 and 53 week interim sacrifices, mean ovary weights and ratios were generally similar in Groups 5 and 6. At the 79 week interim sacrifice, mean ovary weights and ratios were slightly higher in Groups 3 and 4 and higher in Group 6 when compared to the control Group 1. When the ovary weights from one Group 4 and two Group 6 animals were excluded from analyses (because the weights were approximately 6 to 10 times the average), mean ovary weights and ratios were slightly higher in the Group 3 animals while values in all other groups were similar when compared to the control Group 1 at Week 79. For females sacrificed at Week 53, statistically significantly lower mean absolute heart weights were observed for Groups 3 and 5 and statistically significantly lower heart/brain weight ratios were observed for Groups 2 and 3 when compared to the Group 1 control. Mean absolute heart weights and heart/body and heart/brain weight ratios for females were comparable between groups at the
27 week and 79 week interim and the terminal sacrifices. A significantly lower mean brain to terminal body weight ratio was observed for Group 2 males when compared to Group 1 controls after Week 78; however, this value was almost identical to Groups 1 and 2 males at terminal sacrifice. Mean absolute kidney weight values and kidney to brain weight ratios for Group 5 females were significantly lower

than Group 6 values at terminal sacrifice. The other statistically significant difference revealed at terminal sacrifice was a lower mean terminal body weight value for Group 5 females when compared to Group 6 females. No other statistically significant differences were observed in the remaining organ weight data. The effects on the ovary, heart, brain, and kidney are considered incidental and unrelated to treatment due to the lack of dose relationship and/or the absence of effect at subsequent intervals.
- Histopathology incidence and severity:
Interim Sacrifices - Microscopic evaluation of tissues from mice of the Group 1 control, Group 6 sodium control, and Group 5 high-dose groups sacrificed following 26, 52, and 78 weeks of treatment failed to reveal compound-related histomorphologic alterations. The incidence of chronic nephropathy and focal mononuclear infiltrate in the kidneys of the sodium control group were somewhat decreased as compared to the control and monosodium cyanurate groups at Week 53; however, at Week 79, these incidences were comparable between groups. A variety of spontaneous disease lesions and incidental findings was noted and these were of the expected incidence and frequency for mice of this age and strain and occurred without relationship to treatment.
Neoplasms were noted in the tissues examined from the Weeks 52 and 78 sacrifices and consisted of a hepatocellular adenoma in the liver of a Group 6 male (Week 52) and hepatocellular neoplasms in three mice each in Groups 1 and 5 (Week 78); and an alveolar bronchiolar adenoma in the lungs of a Group 6 female (Week 52) and one control male (Week 78).
Terminal Sacrifice -There were no significant treatment-related histopathologic changes in the animals receiving high doses of monosodium cyanurate. Lesions present in Group 5 generally had a similar incidence and severity to Group 1 and/or Group 6 which indicated no specific histopathologic effects of the monosodium cyanurate alone. A variety of spontaneous, geriatric, and incidental lesions was present in all groups at expected incidence and frequency for mice of this age and strain and occurred without significant relationship to treatment.
Microscopic evaluation of tissues from mice (terminal sacrifice and unscheduled deaths) receiving high doses of monosodium cyanurate failed to reveal specific treatment-related histopathologic alterations. These findings are consistent with the results of the 27, 53 and 79 week interim sacrifices. There was an increased incidence of bilateral focal mineralization ("brain sand") in the brain of Group 5 female mice (53%) compared to either Group 1 (33%) or Group 6 (16%). This is a common lesion with a reported incidence of up to 50% in $B_6C_3F_1$ mice and its incidence is influenced by the plane of section taken through the brain (Goodman, al., 1981). Therefore, the study directors did not consider this finding to be treatment related.
In the urinary system, the kidneys from all groups had lesions consistent with chronic nephropathy; however, these lesions were generally minimal to slight. Intratubular microcalculi, present primarily in male mice, were generally scored minimal to slight with little difference between treated and control animals.
In the liver, a variety of neoplastic and nonneoplastic hepatocellular lesions was present with no significant relationship to treatment. Most nonneoplastic liver lesions were generally scored minimal to slight with no evidence of treatment-related hepatotoxicity. The most common findings present in all groups included focal inflammatory cell infiltrates, cytomegaly, sinusoidal cell pigmentation, and individual cell necrosis. Increased hepatocyte vacuolation varied from centrilobular to diffuse and was consistent with fat and/or glycogen and was not associated with hepatocellular degeneration, necrosis, or inflammation. The incidence of hepatocellular adenomas and carcinomas was similar between groups.
In the epididymis of two Group 1, one Group 5, and two Group 6 male mice, there was an increased incidence of a connective tissue tumor designated Sarcoma -Not Otherwise Specified. All of these tumors were generally similar morphologically and were composed of both spindle and oval cells with a foamy pale cytoplasm. Multinucleated giant cells were often present. These tumors appeared similar to histiocytic sarcomas described in the uterus and cervix of female mice.
In the endocrine system, there was a significant decrease in focal pituitary hyperplasia in Group 5 and Group 6 compared to Group 1. The study directors found the significance of this finding to be unclear and of little biologic importance. The incidence of pituitary adenomas was similar between control and treated groups. There was also an increased incidence of focal medullar hyperplasia in Group 5 (5/69) versus Group 1 (1/67). However, in four of the five Group 5 mice, this lesion was minimal (+1) arid this increased incidence was not statistically significant.
There was increased congestion in the mesenteric lymph node in Group 5 and Group 6 versus Group 1 female mice. The study directors found the importance of this finding unclear and of little biologic significance.

CONCLUSIONS

Study Director's Comments: "Based upon the conditions and findings of this study, no definitive treatment-related effects were observed in any of the levels of monosodium cyanurate tested. In addition, monosodium cyanurate was not found to be oncogenetic in mice."

EPA Data Evaluation Report: "Under the conditions of the study, monosodium cyanurate was not oncogenic when administered to male and female $B_6C_3F_1$ mice, at nominal concentrations of 100, 400, 1200, and 5375 ppm in drinking water for 104 weeks. Average compound consumption at the highest dose was 1523 and 1582 mg/kg/ day for males and females, respectively. The highest dose represented the limit of solubility of the test compound. The NOEL for both sexes is the HDT *(highest dose tested)*."

DATA QUALITY

• Reliability: Klimisch Code 1a (Reliable without restriction; comparable to OECD guideline 451)
• Remarks field for Data Reliability: The EPA Data Evaluation Record classifies this study as "Core Guideline"

REFERENCES (Free text):

"104-week oncogenicity study in mice", Hazleton Laboratories America, #2169-101, February 7, 1986, Accession Numbers 261671-81.

EPA Data Evaluation Record, November 1986, MRID 126361.

16d

Repeat Dose Toxicity

TEST SUBSTANCE

• Identity:
Cyanuric acid, monosodium salt; CAS No. 2624-17-2
Sodium dichloro-s-triazinetrione dihydrate; CAS No. 51580-86-0
s-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro-; CAS No. 87-90-1
• Remarks field for Test Substance: All the test materials were white powders. Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/Guideline followed: Comparable to OECD Guideline 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents". The study was designed to evaluate the possible toxicity of the test compounds and to establish dosage levels for a 13 week toxicity study.
• Test type: Repeat dose
• GLP: Yes
• Year: 1980
• Species: Rat
• Strain: CD® (Charles River)
• Route of Administration: Oral, drinking water
• Duration of Test: 59 days
• Doses/Concentration levels:
400, 1200, 2000, and 4000 ppm (cyanuric acid);
400, 1200, 4000, and 8000 ppm (sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione)
• Sex: Male/female
• Exposure period: 59 days
• Frequency of treatment: *ad libitum*
• Control group and treatment: Yes, vehicle (tap water) drinking water
• Post exposure observation period: None
• Statistical Analysis: All statistical analyses compared the treatment groups of each compound with the control group, by sex. Body weights (weeks 4 and 8), water consumption (weeks 4 and 8), hematological, biochemical, and urinalysis parameters (day 59) and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test of homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by the Steel and Torrie. Dunnett's multiple comparison tables were used to judge significance of differences.
• Remarks field for Test Conditions:
- Age at study initiation: males: 84-100 g; females: 84-101 g (approximately 4 weeks old)
- No. of animals per sex per dose: 5/sex/dose (test compound); 10/sex/dose (control)
- Vehicle: water
- Clinical Observations: The rats were observed twice daily, 7 days a week for signs of overt toxicity, moribundity and mortality. Detailed observations were recorded weekly. Moribundity and mortality were recorded on the day noted. Individual body weights were recorded twice weekly. Individual food consumption values were recorded weekly. Water consumption values were recorded three times a week. An extensive list of hematology, clinical chemistry and urinalysis were performed on all rats which survived to the termination of the study.
- Organs examined at necropsy (macroscopic and microscopic):
Macroscopic - All sacrificed rats and rats that died during the study were necropsied and gross lesions, if any recorded. Liver and kidney weights were recorded at necropsy for all sacrificed animals. From all necropsied animals samples of an extensive list of organs and tissues were collected and fixed.
Microscopic - Microscopic examination of ureters, urinary bladders and kidneys was performed for all animals in the control group and the s-triazinetriol, monosodium salt treated groups (group I through V).
Kidney sections from 3 control and 3 high dose animals were also examined using an electron microscope.

RESULTS

• NOAEL (NOEL): ca. 400-1200 ppm
• LOAEL: (LOEL): ca. 2000-2000 ppm
• Toxic response/effects by dose level: see remarks below
• Statistical results: see remarks below
• Remarks field for Results:
- Body weight: Individual body weights were recorded twice weekly. A dose-related decrease in group mean body weights was noted for the 4000 and 8000 ppm groups (males and females) treated with sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione when compared with the control groups. This decrease was statistically significant ($p < 0.01$) for the males at 4 and 8 weeks.
- Food Consumption: Food consumption was recorded weekly and water consumption was recorded three times each week.
A dose-related decrease was noted in average food consumption values for the 4000 and 8000 ppm sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione groups when compared to control groups.
- Water Consumption: A dosage related decrease in mean water consumption was noted in all sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione treated groups when compared to the control group. The decrease in mean water consumption values was statistically significant ($p < 0.01$ or $p < 0.05$) for almost all the groups, at 4 and 8 weeks of study.
- Clinical signs: No compound related signs were noted for the cyanuric acid group. After eight weeks of study, animals in the 4000 and 8000 ppm sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione groups had compound related signs which included: labored breathing, emaciation, accumulation of yellow material on the anogenital regions, decreased defecation, decreased activity and death. For some of the animals in the 8000 ppm groups these signs were evident by week 1 of the study and were followed by death of the animals.
S-TRIAZINETRIOL: All hematology and clinical chemistry values were well within the normal range of historical laboratory control values for this species. Occasional values were statistically significant but the absolute differences were small. The urine values were within the normal range except for the urea nitrogen, which was significantly decreased, in female rats at 1200 ppm and in both male and female rats at 2000 and 4000 ppm, by about 50%. There was no evidence of liver or kidney disease to suggest a pathological event.
SODIUM DICHLORO-S-TRIAZINETRIONE: All hematology and clinical chemistry values are well within the normal range of historical control values for this species. Occasional values were statistically significant, but the absolute differences were small and the numbers of animals surviving for terminal blood and urine sample collection were minimal. Urine volume and urine creatinine were significantly decreased in high-dose males. This may be due to the decrease in water consumption and mean body weight in this group, although this was not true for the corresponding high-dose female groups, which had the same body weight and water consumption effects.
TRICHLORO-S-TRIAZINETRIONE: All hematology and clinical chemistry values well within the normal range of historical control values for this species. Occasional values were statistically significant, but the absolute differences were small and the numbers of animals surviving for terminal blood and urine sample collection were minimal. Urine volume and urine creatinine were significantly decreased in high-dose males. This may be due to the decrease in water consumption and mean body weight in this group, although this was not true for the corresponding high-dose female groups, which had the same body weight and water consumption effects.
- Hematological/Clinical biochemistry findings:
S-TRIAZINETRIOL: All hematology and clinical chemistry values were well within the normal range of historical laboratory control values for this species. Occasional values were statistically significant but the absolute differences were small. The urine values were within the normal range except for the urea nitrogen, which was significantly decreased, in female rats at 1200 ppm and in both male and female rats at 2000 and 4000 ppm, by about 50%. There was no evidence of liver or kidney disease to suggest a pathological event. The study directors concluded that the urea nitrogen results may have been due to some interference with the assay by the test compound or a metabolite.
SODIUM DICHLORO-S-TRIAZINETRIONE: All hematology and clinical chemistry values are well within the normal range of historical control values for this species. Occasional values were statistically significant, but the absolute differences were small and the numbers of animals surviving for terminal blood and urine sample collection were minimal. Urine volume and urine creatinine were significantly decreased in high-dose males. This may be due to the decrease in water consumption and mean body weight for this group, although the high dose females had normal urine volumes and creatinine, while having decreases in water consumption and body weight similar to those seen in the males.
TRICHLORO-S-TRIAZINETRIONE: All hematology ad clinical chemistry values well within the normal range of historical control values for this species. Occasional values were statistically significant, but the absolute differences were small and the numbers of

animals surviving for terminal blood and urine sample collection were minimal. Urine volume and urine creatinine were significantly decreased in high-dose males. This may be due to the decrease in water consumption and mean body weight in this group, although this was not true for the corresponding high-dose female groups, which showed the same body weight and water consumption effects.
- Mortality and Time to Death: Apparent compound-related deaths occurred as early as one week in the 4000 and 8000 ppm sodium dichloro-s-triazinetrione dihydrate and trichloro-s-triazinetrione groups.

		Number Surviving at 59 days/Number Initiated	
Dosage Level (ppm)	Test Compound	Males	Females
0 (Control)			
400	s- triazinetriol, monosodium salt	5/5	5/5
1200	s- triazinetriol, monosodium salt	5/5	5/5
2000	s- triazinetriol, monosodium salt	5/5	5/5
4000	s- triazinetriol, monosodium salt	5/5	5/5
400	sodium dichloro-s-triazinetrione dihydrate	5/5	5/5
1200	sodium dichloro-s-triazinetrione dihydrate	5/5	5/5
4000	sodium dichloro-s-triazinetrione dihydrate	4/5	4/5
8000	sodium dichloro-s-triazinetrione dihydrate	2/5	1/5
400	trichloro-s-triazinetrione	5/5	5/5
1200	trichloro-s-triazinetrione	5/5	5/5
4000	trichloro-s-triazinetrione	4/5	5/5
8000	trichloro-s-triazinetrione	2/5	1/5

- Gross Pathology Incidence and Severity: No gross or microscopic lesions of treatment-related significance were evident in the s-triazinetriol, monosodium salt-treated animals. The few gross and microscopic lesions described showed an equal incidence in control and treated animals. These lesions are common spontaneous lesions and the study directors did not attribute them to treatment.
- Organ Weight Changes: No statistically significant organ weight changes were evident in the s-triazinetriol, monosodium salt, treated rats. The organ weights in the groups treated with chlorinated compounds showed a statistically significant decrease in absolute liver weights at 8000 ppm, which the study directors thought was probably treatment related. The study directors also thought variations in relative organ weights seen in these groups are probably due to the decrease in body-weight gains.
- Histopathology Changes and Severity: Mild and focal inflammatory changes were present in the kidneys of a few animals in each of the experimental groups. Except for these changes there were no histologic abnormalities in the kidneys, ureters, and urinary bladders of the animals. Electron microscope examination revealed no ultrastructural differences between controls and treated animals as to the appearance of various cell organelles or the presence of abnormal precipitates intracellularly, or in tubular lumens and renal interstitium.

CONCLUSIONS

Study Director's Comments: Mild and focal inflammatory changes were present in the kidneys of a few animals in each of the experimental groups. Except for these changes there are no histologic abnormalities in the kidneys, ureters, and urinary bladders of the animals. Histopathologic examination of the kidneys, ureters, and urinary bladders in a 59 Day Dosing Study reveals no differences between the control and treated groups of animals. Compound-related signs were noted in the animal groups receiving 4000 and 8000 ppm of the chlorinated compounds. These signs included: emaciation, decreases in mean body weight, food consumption and water consumption and death. These compound-related effects were caused by the high concentration of chlorine from the test articles in the drinking water of these animal groups. Because of these effects, it was recommended that either the chlorinated compound not be used in the 13-week study or if they were used, the dosage levels be reduced.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restrictions; Comparable to guideline study) (acceptable as a dose ranging study).

• Remarks field for Data Reliability: Does not meet important criteria of OECD Guideline 408 for repeat dose studies i.e., ophthalmological exam, sensory reactivity observation, blood clotting time, extent of histopathology)

REFERENCES (Free text):

Biava, C.; 28-Day Dosing Study in Rats (Extended to a 59-Day Dosing Study) - Revised Report on Histopathological Examination of Urinary Track; Study by International Research and Development Corporation, Mattawan, MI 49071 for FMC Corporation, Monsanto Company, Olin Corporation, ICI Americas Inc. and Nissan Chemical Industries, Ltd.; Study No. 167-150 (IR-80-175); September 12, 1980; MRID 124779.

Biava, Claude; "28 Day Dosing Study in Rats (Extended to 59-Day Study) - Revised Report on Pilot Electron Microscopic Examination of Kidneys from Control and High-Dose Treated Rats"; Study Conducted by the University of California, Department of Pathology; Study No. 167-150; April 15, 1981.

16e

Repeat Dose Toxicity

TEST SUBSTANCE

• Identity: Sodium isocyanurate; CAS No.: 2624-17-1
• Remarks field for Test Substance:
- Purity: 99.7%
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 451 "Carcinogenicity Studies"
• Test type: Carcinogenicity
• GLP (Y/N): Yes
• Year (study performed): 1985 (Study was performed 1981-1983)
• Species: Rat
• Strain: CD® (Charles River)
• Route of administration: Oral, drinking water
• Duration of test: 104 weeks
• Doses/concentration levels: Control (100 male and female), 400 ppm (80 male and 80 female), 1200, 2400, 5375 ppm sodium cyanurate (all 100 male and females), 7768 ppm sodium hippurate (sodium control) (80 males and 80 female)
• Sex: Male/female
• Exposure period: 104 weeks main study; 62 weeks recovery study
• Frequency of treatment: *ad libitum*
• Control group and treatment: vehicle (tap water), Post exposure observation period: Weeks 63-104 for recovery group
• Statistical methods:
Body weights, food consumption, organ weights (absolute and relative) and clinical laboratory values were analyzed using Bartlett's test for homogeneity of variance and analysis of variance (one-way classification). Treatment groups were compared to the control group, and to the sodium control group by sex, using the appropriate t-statistic (equal or unequal variance), as described by Steel and Torrie and Ostle. (Thomas, D. G., Breslow, N., Gart, J. J. Trend and homogeneity analysis of proportions and life table data. Computers and Biomedical Research 10: 373-381, 1977.) Dunnett's multiple comparison tables were used to determine significance. All statistical tests were two-tailed, with $p < 0.05$ and $p < 0.01$ used as levels of significance.
Survival data and data on time to neoplastic lesion were analyzed using the computer program of Thomas, Breslow and Gart. (Thomas, D. G., Breslow, N., Gart, J. J. Trend and homogeneity analysis of proportions and life table data. Computers and Biomedical Research 10: 373-381, 1977.) Statistical procedures included in this program are the Kaplan-Meier and standard methods for computing survival curves, Cox's test for linear trend in proportions, and both Cox's test and Geham-Breslow's generalized Kurskal-Wallis test for comparing survival distributions. Data on time to neoplastic lesion were analyzed for all benign tumors, all malignant tumors, all tumors combined, and for each individual tumor type that appeared in two or more animals in the high dose group. When appropriate, the option for deleting early deaths was also used.
• Remarks field for Test Conditions:
- Age at study initiation: 5 weeks (Males: 87.0 -164.0 g; Females: 72.0 -141.0 g)
- No. of animals per sex per dose: Main study: 80; Recovery study: 20
- Study Design:

Test group	Dose in drinking water (ppm)	Main (24 study months)		Recovery study (not dosed from weeks 63-104)	
		Males	Females	Males	Females
1 Control	0	80	80	20	20
2 (LDT)	400	80	80	--	--
3	1200	80	80	20	20
4	2400	80	80	20	20
5 (HDT)	5375	80	80	20	20
6 Na control	7768	80	80	--	--

The recovery group of animals received drinking water containing test material for 63 weeks and were not dosed thereafter.
- Vehicle: Water
- Clinical observations: Rats were observed twice daily for signs of toxicity, moribundity, and mortality; detailed health examinations were performed weekly. Rats were weighed weekly for 14 weeks and every 2 weeks thereafter. Water consumption values were recorded weekly (the water was provided in lick bottles). Food consumption (individual) was recorded weekly for the first 14 weeks and every other week thereafter. Blood was collected and hematological analyses performed two times before study initiation (nonfasting) from 10 animals/sex and at 6, 12, 18, and 24 months, on 10 animals/sex/group (fasted overnight) and from 10 animals/sex in the recovery groups at 24 months. Urine was collected and urinalyses performed at the same intervals as for blood.
- Organs examined at necropsy (macroscopic and microscopic): All animals that died and that were sacrificed on schedule were subject to gross pathological examination. A full tissue complement was prepared for all animals in the control and high-dose groups. Sections of selected organs (adrenal, heart, kidney, liver, ovary, spleen, testis, ureter and bladder, tissue masses, and gross lesions) were prepared from animals in groups 2, 3, and 4 sacrificed at 6, 12, and 18 months and all animals that died or were sacrificed in extremis. All necropsied animals had other tissues fixed in formalin for possible future examination. In addition, three coronal sections through the head including nasal tissues, nasopharynx, and middle ear were examined in 10 animals/sex in the control and high-dose groups at termination. Organs weighed at necropsy included: Liver, lung, heart, spleen, kidneys, testes & with epididymides, brain, adrenals, thyroids and parathyroids.

RESULTS

• NOAEL (NOEL):
EPA Data Evaluation Record: "…the overall NOEL was 2400 ppm sodium s-triazinetriol (sodium cyanurate monohydrate) based on decreased survival in males and lesions of the urinary tract and heart.
Under the conditions of the study, sodium s-triazinetriol [sodium salt of cyanuric acid] was not oncogenic when administered to Charles River CD-1 rats at levels of 400, 1200, 2400 or 5375 ppm in the drinking water for 2 years. "
• LOAEL (LOEL):
EPA Data Evaluation Record: "The LOEL for the study was 5375 ppm based on decreased survival in males and lesions in the urinary tract and heart…."
• Actual dose received by dose level by sex, if known: Compound intake was calculated from water consumption and analytically determined levels of test compound in water.

Dose Level (ppm)	Average compound consumption (mg/kg/day)	
	Male	Female
0	0	0
400	25.1	41.5
1200	76.4	129
2400	154	266
5375	371	634
7768 (Na control)	60.2	99.4

Female groups consumed 65 to 73 percent more test compound than the corresponding male group (on a mg/kg basis) because of the

increased water consumption smaller body weight of the females.
• Statistical results: See individual discussions below.
• Remarks field for Results:
- Body weight: Group mean body weights of control and treatment animals were similar throughout the study. Mean body weights in both dosed males and females tended to be slightly higher than in negative controls or sodium controls. However, mean food consumption values were similar in all groups. Group mean body weights in recovery groups of females that had previously received 2400 or 5375 ppm were slightly increased when compared to the respective control (14-18 percent) and were increased compared to the respective non-withdrawal group (12-14 percent). In females receiving 1200 ppm, mean body weight in the recovery group was lower than in the non-recovery group. The food consumption values, however, were similar in the female recovery and control groups.
- Food/water consumption: Group mean food consumption values of control and treatment animals were similar throughout the study. There were compound-related increases in water consumption in males and females receiving 2400 and 5375 ppm when compared to negative controls; however, the mean values were comparable to the sodium controls. Thus the increased water consumption in these groups was attributed to a high intake of sodium and a consequent increase in urinary excretion of sodium. The effect was more marked in females than in males. There were no definitive trends in water consumption in the recovery groups of rats; however, mean water consumption between weeks 63 and 104 was lower in the recovery group of rats of both sexes that had previously received 5375 ppm than those continued at this high dose.
- Description, severity, time of onset and duration of clinical signs: There was an increase in incidence of reddish urine in high-dose males during the first year of the study. The incidence of palpable masses was similar in dosed and control groups except for high-dose males and sodium control males during weeks 1-13 when the incidence was higher than in controls; this did not persist at intervals after week 13. No other clinical observations or behavioral effects were considered to be related to dosing. In the recovery groups, clinical findings were similar when compared to the corresponding group that remained on dosing.
- Hematological findings incidence and severity: All hematology parameters in dosed groups were similar to controls at each interval of analysis. Sporadic changes that reached a level of significance ($p < 0.05$) were not consistent over time or with dose and were not considered of toxicologic importance. All values were within the normally expected range.
- Clinical biochemistry findings incidence and severity:
All clinical chemistry parameters in all groups were similar, either when dosed groups were compared with controls or when recovery and non-recovery groups were compared.
In general, the urinalysis results were considered unremarkable. There were sporadic changes in urinary parameters in dosed rats that were significantly different from control values ($p < 0.05$); however, there was no pattern consistent with time or dose and the changes were considered of no toxicologic importance. Copper and zinc levels were generally similar in dosed and control groups at 12, 18, and 24 months. The clinical chemistry results did not indicate any adverse effects on liver, heart, or kidney function as a result of treatment with the test article.
- Mortality and time to death: There was a slight reduction in survival of male rats receiving 5375 ppm when compared to the negative control. The mortality rate was increased in this group during the first year of the study because of urolithiasis and subsequent uremia. Survival was similar in all groups of females. In the recovery groups, at 104 weeks the survival of females that had previously received 1200 or 2400 ppm (7/20 and 9/20, respectively) was somewhat lower than controls (15/20) but survival was similar to control in the group that previously received 5375 ppm (11/20). Survival in male recovery animals was similar in all groups.
- Gross pathology incidence and severity: There were gross lesions in the urinary tract of males receiving 5375 ppm. These were confined primarily to male rats that died in the first year of the study. Hydronephrosis of the kidneys was found in 3/7 high-dose males that died or were sacrificed in extremis between 0-6 months, 4/6 between 6-12 months, and 2/9 sacrificed at 12 months. Calculi (gritty material) were found in the kidneys of 5/7 and 1/6 high-dose males that died or were sacrificed in extremis between 0-6 and 6-12 months, respectively, and in 1/10 and 3/9 high-dose males sacrificed by design at 6 and 12 months, respectively. Hydroureter was found in 2/7 and 4/6 high-dose males that died or were sacrificed moribund between 0-6 and 6-12 months, respectively. None of the male or female rats that were sacrificed at 12, 18 or 24 months or any of the rats that died/sacrificed in extremis during months 12-24 showed any test article related effect. Lesions that occurred in the urinary tract in the second year of the study were reported to be random, and there was no suggestion of a dose-response relationship. The incidence of urinary tract lesions in recovery animals was similar in dosed and control groups and their occurrence was sporadic. The incidence of gross lesions at other sites was sporadic and not compound related.
- Organ weight changes: There were no test article related organ weight variations in the treatment groups at any of the interim or terminal sacrifices. At the 6, 12, and 18 month sacrifices there were only sporadic changes in organ weights that differed significantly ($p < 0.05$) in dosed groups when compared to controls. There were no patterns for effects that were consistent with time or dose. At the 12 month sacrifice, the absolute and relative weights of thyroid and parathyroid were significantly ($p < 0.05$ or 0.01) lower than controls in males receiving 2400 or 5375 ppm. At the 18 month interim sacrifice and at the terminal sacrifice there were no

compound-related changes in organ weights when compared to negative controls. There were some scattered significant differences ($p < 0.05$) when dosed groups were compared to the sodium control; however, most of the differences were small, occurred in the 400 ppm groups, and there were no dose-response trends.
- Histopathology incidence and severity:
Nonneoplastic - There was a compound-related increase in histologic lesions of the urinary system and heart in high-dose males. Most of the lesions occurred in the first 12 months of the study; these were more frequent in high-dose males that died or were sacrificed moribund than in those sacrificed by design at 6 and 12 months. It was reported that the urinary tract lesions were probably all related to calculi; however, calculi were not found histologically and were probably lost in tissue fixation. Urolithiasis in high-dose males was much more frequent than in high-dose females and this was attributed to obstruction of the urethra, which is more likely in males since the urethra is longer than in females and the penile portion is less distensible. The lesions of the heart, acute myocarditis, necrosis, and vascular mineralization were considered secondary to uremia caused by the urinary tract obstructions. Nine of 11 male rats receiving 5375 ppm that died or were sacrificed in extremis in the first year of the study having heart lesions also had calculi in and distension of the bladder at necropsy. The incidence of splenic hemosiderosis was also increased in high-dose males in the first year of the study. There were no compound-related increases in the incidence of lesions at other sites and no increase in heart or urinary tract lesions in rats sacrificed at 18 or 24 months.
Neoplastic - There was no evidence of an oncogenic effect. There were no increases in the incidence of tumors at any site when high-dose groups were compared to controls and no rare tumors occurred in dosed groups.

CONCLUSIONS

Study Director's Comments: "Test article related non-neoplastic lesions were observed only in the urinary tract in males from the 5375 ppm group sacrificed at the 6 and 12 months interims. Test article related heart and urinary tract lesions occurred in 5375 ppm males which died or were sacrificed in extremis or during the first 12 months of study. The only test article related changes in females were in the urinary tract of 5375 ppm animals which died or were sacrificed in extremis during the first 6 to 12 month period of study. The no-effect dosage for both male and female rats was 2400 ppm at 6 and 12 months of study.
In the 18 month interim sacrifice and the 24 months terminal sacrifice, no lesions were detected which could be attributed to treatment with the test article. Therefore, for the last 12 months of study the no effect dosage level was 5375 ppm for both male and female rats. No evidence of a test article related carcinogenic effect was observed in this study. The overall no- effect dose level for the study was 2400 ppm sodium cyanurate for both male and female rats."

EPA Data Evaluation Report: "We agree with the conclusions and interpretations of the report author. The compound-related effect on the urinary system and heart of males receiving 5375 ppm was noted mainly during the first 12 months of the study. This is rather unique since chronic dosing is likely to cause detrimental effects in a progressive manner with time. It appears that the test compound adversely affected a susceptible subset of males that died early in the study; however, the criteria for the susceptibility are not clear in this study. The differences in susceptibility of males and females to urinary tract lesions can be correlated with the differences in anatomy of the urethra. The male urethra is more prone to obstruction by calculi. The incidence of neoplasms found in the study in all groups is what is expected historically for CD rats."

Expert Panel: An Expert Panel was convened to review several toxicity studies conducted on sodium cyanurate and cyanuric acid. The Panel concluded that the rat study discussed herein is the most appropriate for extrapolating to humans. Based on their review, the Panel determined that "under non-emergency conditions, drinking water that contains 1.0 mg/L of the chlorinated isocyanurates which corresponds to an intake of 2.0 mg/day or 0.028 mg/kg/day in adults and 1 mg/day or 0.045 mg/kg/day in children" should be safe for human consumption. The report also notes "using the mean of the highest doses employed in the chronic rat study, 502.5 (i.e., 371 and 634 mg/kg/day in males and females, respectively), the margins of safety (MOS) are nearly 18,000 in adults and >11,000 in children." Also, "sodium dichloroisocyanurate is currently approved at a rate of 10 mg/l of available chlorine, which corresponds to 9.5 mg/l of isocyanuric acid. Under these conditions, this concentration will result in consumptions of 19 mg/day or 0.27 mg/kg/day in adults and 9.5 mg/day or 0.43 mg/kg/day in children. Employing the mean highest dose in the rat study, 502.5 mg/kg/day, results in MOS of >1,800 in adults and >1,100 in children. Gaseous chlorine is currently approved for use, as a disinfecting agent in human drinking water, at a maximum concentration of 30 mg/L (reference). Sodium isocyanurate consumption at levels equivalent to the maximum approved for chlorine [gaseous] results in an MOS of 600 in adults and 367 in children. All of these exposures are clearly and currently accepted MOS multiples."

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: Meets OECD 451 except for blood smear for all animals at 12, 18 months and prior to sacrifice and microscopic examination of tissues from animals that die unscheduled.
The EPA Data Evaluation Record classifies this study as "Guideline" and notes: 'The study was of adequate design to test oncogenicity and chronic toxicity, the conduct was without problems that would compromise the findings, and the reporting was good; summary tables were supported by individual animal data."

REFERENCES (Free text):

"Chronic toxicity and oncogenicity study in rats (IR-80-177) (I80-441)", International Research and Development Corp., IRDC study # 167-157 (IR-80-177) (I80-441), October 30, 1985.

Cohen, S. M., McKinney, L., Wagner, B. M., Weil, C., Goodman, J. I., Lotti, M., Portoghese, P., Bernard, B. K., An Evaluation of the Long-Term Toxicity/Carcinogenicity of Sodium Isocyanurate, unpublished report to Occidental Chemical Corporation, February 25, 1999, provided to U.S. EPA and assigned MRID No. 44834403.

EPA Data Evaluation Record, November 1986, MRID 126362.

17a

Reproductive Toxicity

TEST SUBSTANCE

• Identity: sodium cyanurate monohydrate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- 77.05% cyanuric acid
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline for Two-Generation Reproduction Toxicity (No. 416)
• Type: Reproductive toxicity
• GLP: Yes
• Year (study performed): 1982 - 1984
• Species: Rat
• Strain: Charles River CD®
• Route of administration: Oral, drinking water
• Doses/concentration levels: 0, 400, 1200, 5375 ppm
• Sex: Male/female
• Control group and treatment: Two; vehicle (tap water) and sodium (8056 ppm sodium hippurate)
• Frequency of treatment: *ad libitum*
• Duration of test: 103 weeks
• Premating exposure period for males: F_0 at least 100 days; F_1 at least 120 days; F_2 at least 120 days
• Premating exposure period for females: F_0 at least 100 days; F_1 at least 120 days; F_2 at least 120 days
• Statistical methods: Analysis compared the treated groups to the control and sodium control groups and the control and sodium control groups were compared to each other. The parental body weights by sex were analyzed by one-way analysis of variance, Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances). Significant differences were determined using Dunnett's multiple comparison tables. In all generations, analyses were conducted at one week prior to the first ("a") mating, one week prior to the second ("b") mating and at termination of the generation. In addition, analyses were conducted at initiation of the F_1 and F_2 generations.
Male and female fertility indices were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test.
The proportion of live pups at birth per total number born and the survival indices at lactation days 4, 7, 14 and 21 were compared by the Mann-Whitney U-test.
The mean numbers of live born pups per litter and mean body weights of pups were analyzed by one-way analysis of variance, Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances). Significant differences were determined using Dunnett's multiple comparison tables.
Organ weights (adrenal, brain, heart, kidney, liver, ovary, spleen, testis/epididymis and thyroid/parathyroid) by sex were analyzed by one-way analysis of variance, Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal or unequal variances). Significant differences were determined using Dunnett's multiple comparison tables.
• Remarks field for Test Conditions:
- Test animals:
F_0: 12 male and 24 female per dose group, dosing began at 36 days of age
F_1 and F_2: 12 male and 24 female per dose group, dosing began at 21 days of age
- Test design: Based on body weight data, animals were divided into two control and three test groups of 12 males and 24 females each to become the F_0 parents. After administration of the test material for at least 100 days, the F_0 parental rats were mated on a 1:2 male to female basis to produce the F_1 offspring. The day of mating was designated as day 0 of gestation. A minimum of 14 days after weaning of the F_{1a} litters, F_0 females were mated again (to different males) to produce the F_{1b} offspring. Pups from the F_{1b} litters were selected at random to become parents for the next generation. After a minimum of 120 days of test material administration the

F_{lb} parents were mated twice, as described above, to produce "a" and "b" litters, and the F_{2b} parents were mated once to produce the F_{3a} offspring.
Offspring from the F_{la} and F_{2a} generations were sacrificed on lactation day 21, necropsied, and discarded. At weaning, 12 male and 12 female F_3 pups per group were randomly selected for continued treatment for another 4 weeks. They were then sacrificed and necropsied, and designated tissues were collected. The remaining F_3 weanlings were sacrificed and necropsied, and the carcasses were preserved.
- Vehicle: tap water
- Mating procedures: (length of cohabitation, proof of pregnancy): 7-14 days; 1:2 male/female ratio per cage; vaginal smears and/or vaginal plug as sign of copulation
- Standardization of litters (yes/no and if yes, how and when): reduced to 10 pups on lactation day 4
- Parameters assessed during F_0 and F_1:
Clinical observations performed and frequency: All animals were examined twice daily for mortality and clinical signs. Detailed physical examinations were conducted weekly on the parental rats and on the pups after weaning. All pups were examined for gross deformities on days 0, 4, 7, 14, and 21 of lactation.
Individual body weights were recorded weekly for parental rats and for the F_3 pups that were retained. Females were weighed on days 0, 6, 15, and 20 of gestation and days 0, 7, 14, and 21 of lactation. Pups were individually weighed on days 0, 4, 7,14 and 21 of lactation.
Parental food consumption was measured once weekly from initiation to termination of each generation, except during mating.
Individual water consumption data were recorded weekly for parental rats (except during mating) and for F_3 pups. Water consumption was measured on a per cage basis during mating.
Male and female fertility indices and the length of gestation were tabulated. Any abnormalities in nesting and nursing behavior and difficulties at parturition were recorded.
Litter parameters assessed included mean numbers of live and stillborn pups per litter, pup survival to weaning, pup body weights, and clinical observations.
- Parameters assessed during study F1 and F2:
Clinical observations performed and frequency: See above
Organs examined at necropsy (macroscopic and microscopic):
Organ Weights - All terminally necropsied animals: Adrenal, brain, heart, kidney, liver, ovary (2), spleen, testis with epididymis (2), thyroid.
Histopathology - Microscopic examination was performed on gonads and gross lesions of F_0, F_1 and F_2 parents. F_{1b} and F_{2b} pups (5/sex from control, high dose and sodium control) and F_3 (5/sex/group) as follows: adrenals, aorta, bone (tibia with marrow), brain (longitudinal section), colon, duodenum, esophagus, eyes, heart, ileum, jejunum, kidney, liver, lungs, lymph node (mesenteric), mammary gland, ovaries, pancreas, pituitary, peripheral nerve, prostate, salivary gland, seminal vesicles, skeletal muscle, skin (with cervical lymph node), spinal cord, spleen, stomach, testes (with epididymis), thymus, thyroid (with parathyroids), trachea, urinary bladder, uterus, cervix, all other gross lesions, tissue masses, ureter.

RESULTS

• NOAEL: 5375 ppm for reproductive and offspring effects; 1200 ppm for adult toxicity
Study Director: "Therefore, a concentration of 5375 ppm was considered a "no-effect" level for the administration of s-triazinetriol with regard to reproductive and litter parameters in this study."
EPA Data Evaluation Record: "The …NOEL for adult toxicity of s-triazinetriol (Na salt of cyanuric acid) administered in drinking water to rats for three generations are assessed as …1200 ppm, respectively. These effects are based on compound related incidence of calculi in the urinary bladders of high dose F_2 females. The test material did not produce any consistent adverse effects with regard to reproductive parameters or offspring toxicity. Therefore, 5375 ppm is assessed as the NOEL for reproductive and offspring effects…."
• LOAEL:
EPA Data Evaluation Record: "The LOEL… for adult toxicity of s-triazinetriol (Na salt of cyanuric acid) administered in drinking water to rats for three generations are assessed as 5375 ppm, the highest dose tested… These effects are based on compound related incidence of calculi in the urinary bladders of high dose F_2 males. The test material did not produce any consistent adverse effects with regard to reproductive parameters or offspring toxicity. Therefore, …for reproductive and offspring effects; the LOEL could not be determined."
• Actual dose: Test material dosing solutions were within ± 10% of the nominal concentration. Sodium control dosing solutions ranged from 87 to 110% of the nominal concentration.

Test compound consumption of parental males, mg/kg/day, at selected intervals.							
		Week of study					
Generation	**Dose (ppm)**	**0**	**8**	**16**	**24**	**34**	**Mean**
F_0	400	92	40	34	27	26	44
	1200	288	126	111	82	82	138
	5375	1333	554	481	357	334	612
	Na+ controls	1780	903	716	577	606	916
F_1		**33**	**43**	**53**	**63**	**72**	**Mean**
	400	126	43	37	35	26	53
	1200	316	117	111	93	75	142
	5375	1697	495	453	381	305	666
	Na+controls	2409	829	784	701	528	1050
		71	**79**	**87**	**95**	**103**	**Mean**
F2	400	70	42	39	37	27	44
	1200	150	117	96	104	76	109
	5375	935	578	479	455	369	563
	Na+ controls	1415	705	736	780	708	869

Test compound consumption of parental females, mg/kg/day, at selected intervals.							
		Week of study					
Generation	**Dose (ppm)**	**0**	**8**	**16**	**24**	**34**	**Mean**
F_0	400	93	63	35	45	53	58
	1200	273	182	175	134	156	184
	5375	1290	993	864	721	748	769
	Na+ controls	1836	1133	1242	923	948	1216
F_1		**33**	**43**	**53**	**63**	**72**	*Mean*
	400	115	57	52	58	43	65
	1200	355	195	166	164	130	202
	5375	1638	801	804	841	617	450
	Na+controls	2374	1320	1330	1386	1112	1504
.		**71**	**79**	**87**	**95**	**103**	*Mean*
F2	400	75	66	62	71	47	64
	1200	232	200	219	234	132	203
	5375	1003	1047	1134	889	783	971
	Na+ controls	1537	1360	1524	1400	1064	1377

• Parental data:
Mortality and Clinical Observations - Low incidences of death or early sacrifice due to moribundity were reported for all generations. No compound-related effect on survival was indicated. General appearance and behavior, including nesting and nursing behavior, were not affected by compound administration. A total of seven F_0 parents failed to survive to scheduled sacrifice; two control, two high-dose and one sodium control male(s) died on study. In addition, one male each in the control and high dose groups were sacrificed prior to termination of the generation. The deaths and sacrifices occurred between study weeks 11 and 34. Six F_1 parents died on study; one male each in the high-dose and sodium control groups and one female each in the control, low- and mid-dose and sodium control groups. Additionally, one control group F_1 female was sacrificed in extremis. Mortality and unscheduled sacrifices occurred between weeks 38 and 63. Five F_2 parents did not survive to scheduled sacrifice; two each in the low- and high-dose groups and one in the sodium control group. Deaths occurred between weeks 69 and 100.
Antemortem observations and necropsy findings of these and the animals that survived to scheduled sacrifice did not indicate a biologically significant effect on survival, appearance or behavior including nesting and nursing behavior.

Body Weights - Evaluation of parental body weight data revealed no cross-generational treads indicative of a test article effect on this parameter. During the first generation, slight (non-significant) increases in body weight were seen in the high-dose (5375 ppm) and

sodium control males. In contrast, during the second generation, body weight inhibition was reported for the high-dose and sodium control males and females and mid-dose (1200 ppm) females. Throughout the third generation, low-dose (400 ppm) male and female body weights were generally higher than the control values. Since no cross-generation trends were apparent, the study directors concluded that parental body weights were not affected by administration of s-triazinetriol at 5375 ppm or less or of sodium hippurate at 8056 ppm. Maternal weights during gestation and lactation were similarly unaffected.

Food Consumption - The food consumption of treated and sodium control parents did not differ significantly from those of the corresponding control group throughout the study.

Water Consumption - Increased water consumption was noted for high-dose females in all generations. In the F_1 and F_2 generations, water consumption was also increased for the sodium control females when compared to the control group. The study directors stated that a definitive compound effect on water consumption was equivocal. No consistent treatment-related effect was apparent for the male test groups. The findings with respect to female water consumption in the high-dose group had no biologically detrimental effect on the reproductive potential of the parents or on the growth and development of the offspring.

Gross Pathology, Organ Weights and Histopathology - No compound-related gross necropsy or histopathological findings or organ weight changes were noted for F_0 or F_1 parents. In the F_2 generation, an increased incidence of calculi in the urinary bladder was noted in high-dose males: 5 of 12 males were affected. This finding was not noted in any other F_2 group, and the study directors considered this a compound-related finding. The urinary bladders of three of these males had epithelial hyperplasia or chronic cystitis, which was considered secondary to chronic irritation from the calculi. The findings with respect to the postmortem urinary bladder findings in high-dose F_2 males had no biologically detrimental effect on the reproductive potential of the parents or on the growth and development of the offspring.

• Offspring toxicity:
Reproductive and Litter Effects - Treatment with s-triazinetriol at concentrations of 5375 ppm or less did not induce significant fluctuations in reproductive or litter parameters. Generally, fertility indices, gestation lengths, litter size, pup survival to weaning and pup weights at parturition and throughout lactation of the treated groups (including the sodium control group) were comparable to those of the corresponding controls. Dystocia or behavioral abnormalities during nesting and nursing were not observed. A generalized reduction in female fertility indices occurred during both breedings of the second generation. These values were similar to the low values of the historical control range. Corresponding mating indices, reflective of the occurrence of copulation were generally considerable greater than the fertility indices. This indicated between 2 and 11 sterile matings in each group per breeding. The lack of both dose-related and cross generational trend disassociated these findings from treatment with s-triazinetriol. A specific cause(s) of inhibited fertility among second generation parents was not readily discernible.

The number of viable pups at birth was decreased at the mid-dose level for the F_{2a} and F_{2b} litters and in high-dose F_{2b} litters. In the third generation, pup body weights for the dose groups, and values for the sodium control group were lower than the controls throughout lactation. The study directors concluded that these findings were not biologically significant because they were not "consistently evident."

No test article related macroscopic changes, organ weight variations or microscopic changes were evident in the F_0, F_{1b}, F_1, F_{2b} or F_3 rats that were subjected to postmortem evaluation. The only finding considered related to the test article was an increased incidence of calculi in the urinary bladder in the 5375 ppm F_2 males. The urinary bladders of three of these males had epithelial hyperplasia or chronic cystitis, which was considered secondary to chronic irritation from the calculi. No further test article related macroscopic, microscopic or organ weight changes were observed among the F_2 rats.

The findings with respect to female water consumption in the high-dose group and postmortem urinary bladder findings in high-dose F_2 males had no biologically detrimental effect on the reproductive potential of the parents or on the growth and development of the offspring.

Gross necropsy of pups that died and of weanlings at scheduled sacrifice revealed no compound-related findings with respect to weanling malformations, variations, or pathological findings.

Retained F_3 Weanlings - Survival, general appearance and behavior, body weight, food and water consumption data of the F_3 pups retained for 4 weeks following weaning were similar among all groups: no compound-related effects were evident. Gross necropsy findings, organ weights, and microscopic findings were also comparable for all study groups.

• Statistical results, as appropriate: N/A
• Remarks field for Results: See preceding paragraphs describing results.
- Food/water consumption: No test compound related effects were observed on food consumption. See preceding paragraphs for discussion of water consumption.
- Description, severity, time of onset and duration of clinical signs: No test compound related effects were observed.
- Fertility index (pregnancies/matings): See preceding paragraphs describing results.
- Precoital interval (w/number of days until mating and number of estrous periods until mating): Not reported.
- Duration of gestation (calculated from day 0 of pregnancy): No test compound related effects were observed in any of the generations.
- Gestation index (live litters/pregnancies): No test compound related effects were observed in any of the generations.
- Mortality: See preceding paragraphs describing results.
- Gross pathology incidence and severity: No compound-related gross necropsy were noted for F_0 or F_1 parents. No test compound related effects. See preceeding paragraphs describing results.
- Organ weight changes: No compound-related organ weight changes were noted for F_0 or F_1 parents.
- Histopathology incidence and severity: No compound-related histopathological findings were noted for F_0 or F_1 parents.
- Offspring toxicity F_1 and F_2: See preceding paragraphs describing results.
- Viability index (pups surviving 4 days/total births): See preceding paragraphs describing results.

CONCLUSIONS

Study Director's Comments: "Therefore, a concentration of 5375 ppm was considered a "no-effect" level for the administration of s-triazinetriol with regard to reproductive and litter parameters in this study."

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guideline study)
• Remarks field for Data Reliability: Would appear to meet OECD 416 if number of pregnant animals per dose group is approximately 20. The EPA Data Evaluation Record classifies this study as "Core Minimum".

REFERENCES

Three generation reproduction study in rats with sodium salt of cyanuric acid (s-triazinetriol). D. Aldridge, J.L. Schardein, M. Blair, J.R. Kopplin and W.R. Richter, IRDC Inc. 497-001, May 10, 1985.

EPA Data Evaluation Record, March 1986, MRID 150286.

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Developmental Toxicity/Teratology

TEST SUBSTANCE

• Identity: Monosodium cyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- 99.7% pure
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 414 "Teratogenicity"
Pregnant female rats were administered the test material by oral gavage on Days 6 to 15 of pregnancy. The dams were observed twice daily during the treatment period with body weights being recorded every third day beginning on Day 6. On Day 20 of gestation, the surviving dams were necropsied and their fetuses removed by cesarean section. All fetuses were weighed, measured, sexed and examined for external abnormalities. Approximately one-half of the fetuses were fixed in Bonn's fixative and examined for abnormalities by razor blade sectioning. The remaining fetuses were prepared for skeletal examination.
• GLP: Yes
• Year (study performed): 1981
• Species: Rat
• Strain: COBS® CD® (Charles River)
• Route of administration: oral (gavage)
• Doses/concentration levels: 200, 1000, 5000 mg/kg/day
• Sex: Female
• Exposure period: Days 6 through 15 of gestation
• Frequency of treatment: Single daily dose
• Control groups and treatment: Four; Untreated; Vehicle Control: 4% aqueous Carboxymethyl cellulose; Low Sodium Hippurate, anhydrous, 1118 mg/kg/day; High Sodium Hippurate, anhydrous, 5590 mg/kg/day
• Duration of test: Through Day 20 of gestation
• Statistical methods:
All statistical analyses compared the treatment groups and the sodium control groups to both the vehicle control group and the untreated control group with the level of significance at $p < 0.01$. In addition, all, statistical analyses compared the untreated control group with the vehicle control group at the same level of significance.
The male to female fetal sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of differences.
The number of early and late resorptions and postimplantation losses were compared by the Mann-Whitney U-test as described by Siegel and Weil to judge significance of differences.
The mean number of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.
• Remarks field for Test Conditions:
- Age at study initiation: 12 weeks (219 to 288 gms)
- Number of animals per dose per sex: 25 pregnant females
- Vehicle: 4% aqueous carboxymethyl cellulose
- Clinical observations: Prior to treatment, the dams were observed twice daily for mortality and overt changes in appearance and behavior. They were observed twice daily for mortality and once daily for clinical signs of toxicity on days 6 through 20 of gestation. A gross necropsy was performed on all rats not surviving to scheduled sacrifice in order to determine the cause of death, if possible.
- Body Weights: Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 15, 18 and 20.
- Mating procedures: One male and one female were placed together for mating. Day 0 of gestation was designated as the day on which sperm was detected by vaginal smear. Females were housed individually during gestation.

- Parameters assessed during study: Body weights of the dams were recorded on days of 0, 6, 9, 12, 15, 18 and 20 of gestation.
- Organs examined at necropsy:
Surviving females were sacrificed by carbon dioxide inhalation on gestation day 20. The uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and nonviable fetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy. Maternal tissues were preserved in 10% neutral buffered formalin for microscopic examination only when deemed necessary by gross findings.
All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed, measured (crown-rump length) and individually numbered and tagged for identification. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning as described by Wilson. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson for subsequent skeletal examination. Fetal findings were classified as malformations or genetic or developmental variations.

RESULTS

• NOAEL maternal toxicity: >5,000 mg/kg/day
No toxic and/or pharmacologic effects related to the test material were observed at doses of up to 5,000 mg/kg/day
• NOAEL developmental toxicity: >5,000 mg/kg/day
Neither teratogenic nor fetotoxic effects related to the test material were observed at doses of up to 5,000 mg/kg/day
• Actual dose received by dose level by sex if available: N/A
• Fetal/Maternal data:
• Statistical results, as appropriate: N/A
• Remarks field for Results:
- Mortality and day of death: Eleven females in the high dose sodium control group (5590 mg/kg/day sodium hippurate) died between days 8 and 16 of gestation. Postmortem observations included matted haircoat in the anogenital, abdominal and/or nasal region, red or black matter in the ocular, abdominal, limbs or nasal area and/or red matter in or around the vagina. No deaths occurred in any of the other control or experimental groups.
- Number pregnant per dose level: There were no significant differences in the number of pregnant animals amongst the various dose groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Number aborting: There were no significant differences in the number of animals which aborted amongst the various dose groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Number of resorptions, early/late if available: There were no significant differences in the number of animals which aborted amongst the various dose groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Number of implantations: There were no treatment-related or statistically significant differences in the mean number of total implantations in the test article groups when compared to the vehicle control and untreated control groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Pre and post implantation loss, if available: There were no test compound related effects on postimplantation losses. In both sodium control groups there was a dose-related increase in mean postimplantation loss when compared to the vehicle control and the untreated control groups, with the value in the high dose sodium control group increased when compared to the historical control data. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Number of corpora lutea (recommended): There were no treatment-related or statistically significant differences in the mean number of corpora lutea in the test article groups when compared to the vehicle control and untreated control groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
- Duration of Pregnancy: No significant differences in the duration of pregnancy was reported amongst the various dose groups.
- Body weight:
Maternal - In the test article treated groups, maternal body weight gain during the treatment period and over the entire gestation period were comparable to the vehicle control and the untreated control groups at dosages of 5000 mg/kg/day and less. The mean maternal body weight gain in the high dose sodium control group was slightly decreased over the entire gestation period when compared to the vehicle control and the untreated control groups.
Fetal - There were no treatment-related or statistically significant differences in the mean fetal body weights in the test article groups when compared to the vehicle control and untreated control groups. There was a marked decrease in mean fetal body weight in the high dose sodium control group when compared to the vehicle control and the untreated control groups. No similar effect was noted in the low dose sodium control group.

- Description, severity, time of onset and duration of clinical signs: In the test article treated groups, maternal appearance and behavior during the treatment period and over the entire gestation period were comparable to the vehicle control and the untreated control groups at dosages of 5000 mg/kg/day and less.
In the high-dose sodium control group, there was an increased incidence of matted haircoat in the anogenital region, extreme resistance to dosing and/or red or black matter in the ocular region in the rats in the high dose sodium control group when compared to both the vehicle control and untreated control groups.
- Gross pathology incidence and severity: In dams, no test compound related effects were seen at necropsy. A treatment related increase in the incidence of reddened stomach mucosa, stomach distended and containing reddish-brown or greenish-tan liquid and/or fluid or mucoidal material in the intestines was observed at necropsy in the high dose sodium control group dams.
- Organ weight changes, particularly effects on total uterine weight: No test compound related effects were seen on uterine weight. A decrease in mean gravid uterus weight were observed in the adult females in the high dose sodium control group when compared to the vehicle control and untreated control groups.
- Fetal data:
Litter size and weights: There were no treatment-related or statistically significant differences in the fetal body weights amongst the various groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section
Number viable (number alive and number dead):There were no treatment-related or statistically significant differences in the mean number of viable fetuses in the test article groups when compared to the vehicle control and untreated control groups.
There was a slight decrease in the mean number of viable fetuses in the high dose sodium control group when compared to both control groups. The remaining Cesarean section parameters in both sodium control groups were similar to the vehicle control and the untreated control groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section
Sex ratio: There were no treatment-related or statistically significant differences in the fetal sex distribution in the test article groups when compared to the vehicle control and untreated control groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section.
Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: There were no treatment-related or statistically significant differences in the crown rump length in the test article groups when compared to the vehicle control group and the untreated control group. In the high dose sodium control group, the mean fetal crown rump length was decreased when compared to the vehicle control, and untreated control groups. See summary table (below) of Maternal and Fetal Observations at Cesarean Section
There was no dose-related or statistically significant difference in the total number of litters with malformed fetuses in the monosodium cyanurate groups or the low dose sodium control group when compared to both the vehicle control and the untreated control group. However, the total number of fetuses with malformations was increased in the monosodium cyanurate groups when compared to both the vehicle control and the untreated control groups. In the high dose monosodium cyanurate group this increase was due to seven fetuses from one litter which had both bent ribs and bent limb bones. Since these malformations were present in only one litter they were judged to be sporadic rather than an effect of treatment. An increased incidence of bent ribs was noted in fetuses in the low and mid dose monosodium cyanurate groups when compared to the vehicle control and untreated control groups. However, this increase did not occur in a dose- related pattern since the malformation was present in seven and three fetuses in the low and mid dose groups, respectively. The remaining malformations occurred with comparable frequency in the monosodium cyanurate, vehicle control and untreated control groups. In the high dose sodium control group an increased incidence of malformations in fetuses (and litters), due primarily to an increased occurrence of bent ribs, was observed when compared to the vehicle control, and untreated control group. When compared to the historical control data the incidence of bent ribs in this group was markedly increased.
In the high dose test article group (5000 mg/kg/day monosodium cyanurate) the percentage of fetuses with the variations sternebrae #1, 2, 3 and/or 4 unossified or vertebrae reduced in ossification was increased when compared to the vehicle control group, the untreated control group and the historical control data. However, since all fetuses with this malformation in the high dose monosodium cyanurate group were restricted to one litter, this difference from the control, groups was not considered to be caused by treatment. All other variations present in fetuses in the monosodium cyanurate groups occurred as single instances and/or were within the range of the historical control data. The incidence of fetuses with sternebrae #5 and/or #6 unossified, other sternebrae unossified, entire sternum unossified, hyoid unossified, skull reduced in ossification or vertebrae reduced in ossification was increased in the high dose sodium control group when compared to the vehicle control group, the untreated control group and the historical control data. In addition, 7th cervical rib(s), 14th rudimentary rib(s), major vessel variations or renal papillae not developed and/or distended ureter occurred with greater frequency in fetuses in the high dose sodium control, group when compared to the vehicle control or the untreated control group. No similar overall increase in genetic or developmental variations was observed in the low dose sodium control group.
EPA Data Evaluation Record - The only treatment-related increase in fetal abnormalities occurred in the high-dose sodium controls. Since 11 of the 25 treated females died, the fetal effects can be most easily explained as secondary to maternal toxicity in the survivors. The increased number of abnormalities were mainly “bent ribs”. A small, non-dose related increase in the number of

fetuses with bent ribs was observed in the cyanurate treated groups. The fact that this did not appear to be treatment related was also supported by the fact that all 7 pups with bent ribs in the high-dose group were in the same litter.

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section

	0 (Vehicle Control)			0 (Untreated Control)		
	No.	%	S.D.	No.	%	S.D.
Animals on study:	25	-	-	25	-	-
Animals that were gravid:	24	96.0	-	24	96.0	-
Animals that died:	0	0.0	-	0	0.0	-
Nongravid:	0	0.0	-	0	0.0	-
Gravid:	0	0.0	-	0	0.0	-
Animals that aborted/delivered:	0	0.0	-	0	0.0	-
Animals examined at Cesarean Section:	25	100.0	-	25	100.0	-
Nongravid:	1	4.0	-	1	4.0	-
Gravid:	24	96.0	-	24	96.0	-
Dams with resorptions only:	0	0.0	-	1	4.2	-
Dams with viable fetuses:	24	100.0	-	23	95.8	-
Viable fetuses/dam:	14.4	-	±2.70	13.7	-	±3.55
Postimplantation loss/dam:	0.8	-	±0.79	0.8	-	±0.79
Total Implantations/dam:	15.1	-	±2.17	14.4	-	±3.37
Corpora lutea/dam:	17.2	-	±2.79	16.1	-	±2.92
Group mean preimplantation loss (%) [a]:	-	11.9	-	-	10.4	-
Group mean preimplantation loss (%) [b]:	-	5.0	-	-	5.2	-
Fetal sex distribution – male:	159	46.1	-	173	52.7	-
- female:	186	53.9	-	155	47.3	-
Mean fetal body weight (grams):	3.6	-	±0.44	3.6	-	±0.23
Mean crown rump length (cm):	3.7	-	±0.16	3.8	-	±0.14

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section (200, 1000, 5,000 ppm)

	Monosodium Cyanurate (mg/kg/day)								
	200			1000			5000		
	No.	%	S.D.	No.	%	S.D.	No.	%	S.D.
Animals on study:	25	-	-	25	-	-	25	-	-
Animals that were gravid:	25	100.0	-	21	84.0	-	25	100.0	-
Animals that died:	0	0.0	-	0	0.0	-	0	0.0	-
Nongravid:	0	0.0	-	0	0.0	-	0	0.0	-
Gravid:	0	0.0	-	0	0.0	-	0	0.0	-
Animals that aborted/delivered:	0	0.0	-	0	0.0	-	0	0.0	-
Animals examined at Cesarean Section:	25	100.0	-	25	100.0	-	25	100.0	-
Nongravid:	0	0.0	-	4	16.0	-	0	0.0	-
Gravid:	25	100.0	-	21	84.0	-	25	100.0	-
Dams with resorptions only:	0	0.0	-	0	0.0	-	0	0.0	-
Dams with viable fetuses:	25	100.0	-	21	100.0	-	25	100.0	-
Viable fetuses/dam:	14.1	-	±2.00	14.7	-	±1.62	14.2	-	±3.32
Postimplantation loss/dam:	0.6	-	±0.58	0.6	-	±0.86	0.7	-	±1.07
Total Implantations/dam:	14.6	-	±1.68	15.3	-	±1.62	14.9	-	±3.17
Corpora lutea/dam:	15.8	-	±2.43	16.7	-	±1.66	16.4	-	±3.19
Group mean preimplantation loss (%) [a]:	-	7.3	-	-	8.4[e]	-	-	5.3	-
Group mean preimplantation loss (%) [b]:	-	3.8	-	-	4.0	-	-	4.6	-
Fetal sex distribution – male:	179	50.9	-	160	51.8	-	165	46.3	-
- female:	173	49.1	-	149	48.2	-	191	53.7	-
Mean fetal body weight (grams):	3.5	-	±0.29	3.6	-	±0.26	3.5	-	±0.40
Mean crown rump length (cm):	3.7	-	±0.13	3.7	-	±0.14	3.7	-	±0.17

Summary of Group Mean Maternal and Fetal Observations at Cesarean Section

	Sodium Hippurate (mg/kg/day)					
	1118			5590		
	No.	%	S.D.	No.	%	S.D.
Animals on study:	25	-	-	25	-	-
Animals that were gravid:	24	96.0	-	25	100.0	-
Animals that died:	0	0.0	-	11	44.0	-
Nongravid:	0	0.0	-	0	0.0	-
Gravid:	0	0.0	-	11	100.0	-
Animals that aborted/delivered:	0	0.0	-	0	0.0	-
Animals examined at Cesarean Section:	25	100.0	-	14	56.0	-
Nongravid:	1	4.0	-	0	0.0	-
Gravid:	24	96.0	-	14	100.0	-
Dams with resorptions only:	0	0.0	-	1	7.1	-
Dams with viable fetuses:	24	100.0	-	13	92.9	-
Viable fetuses/dam:	13.9	-	±2.58	12.6	-	±5.29
Postimplantation loss/dam:	1.3	-	±2.05	2.2	-	±3.70
Total Implantations/dam:	15.1	-	±1.39	14.9	-	±3.96
Corpora lutea/dam:	16.6	-	±2.00	17.9	-	±3.55
Group mean preimplantation loss (%) [a]:	-	9.0	-	-	16.8	-
Group mean preimplantation loss (%) [b]:	-	8.3	-	-	14.9	-
Fetal sex distribution – male:	173	52.0	-	94	53.1	-
- female:	160	48.0	-	83	48.9	-
Mean fetal body weight (grams):	3.7	-	±0.40	3.0[c,d]	-	±0.61
Mean crown rump length (cm):	3.8	-	±0.16	3.5	-	±0.24

[a] $\frac{\text{Total No. Corpora Lutea} - \text{Total No. Implantations}}{\text{Total No. Corpora Lutea}} \times 100 = \text{Group Mean Preimplantation Loss (\%)}$

[b] $\frac{\text{Total No. Implantations} - \text{Total No. Viable Fetuses}}{\text{Total No. Implantations}} \times 100 = \text{Group Mean Preimplantation Loss (\%)}$

[c] Significantly different from the vehicle control group mean; $p < 0.01$

[d] Significantly different from the untreated control group mean; $p < 0.01$

[e] Value does not include dam #92289 with an erroneous number of corpora lutea

S.D. – Standard deviation

- Not applicable

CONCLUSIONS

Study Director's Comments: "Monosodium cyanurate did not produce a teratogenic response when administered orally by gavage to rats at a dose level of 5000 mg/kg/day or less. Sodium hippurate did not produce a teratogenic response when administered orally by gavage at a dose level of 1118 mg/kg/day. Although treatment with sodium hippurate at a dose level of 5590 mg/kg/day was linked with an increased total incidence of malformations this increase did not parallel that commonly observed with classic teratogenic agents."

EPA Data Evaluation Record: "Teratogenic and/or fetotoxic effects were not demonstrated in rats at doses of monosodium cyanurate up to 5000 mg/kg/day (days 6 through 15 of gestation). No toxic and/or pharmacologic effects were observed in the dams at any dose."

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restrictions; Comparable to guideline study)
• Remarks field for Data Reliability: The EPA Data Evaluation Record classifies this study as "Guideline."

REFERENCES (Free text):

"Teratology Study in the Rat with Monosodium Cyanurate", International Research and Development Corporation, 167-159, May 14, 1982.
EPA Data Evaluation Record, August 1982, MRID 105168.

18b

Developmental Toxicity/Teratology

TEST SUBSTANCE

• Identity: Monosodium isocyanurate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- >99.0 % pure
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Comparable to OECD Guideline 414 "Teratogenicity"
Artificially inseminated white rabbits were administered the test material by oral gavage on Days 6 to 18 of gestation. The dams were observed daily during the treatment period. Body weights were recorded every third day beginning on Day 6. On Day 29 of gestation, the surviving dams were necropsied and their fetuses removed by cesarean section. All fetuses were weighed, measured, sexed, examined for skeletal, external and soft tissue abnormalities.
• GLP: Yes
• Year (study perfomed): 1990
• Species: Rabbit
• Strain: New Zealand White (Hazleton Research Products)
• Route of administration: oral (gavage)
• Doses/concentration levels: 0, 50, 200, 500 mg/kg/day
• Sex: Female
• Exposure period: Days 6 through 18 of gestation
• Frequency of treatment: Single daily dose
• Control groups and treatment: One; Untreated, Vehicle Control: 1% aqueous Carboxymethyl cellulose
• Duration of test: Through Day 29 of gestation
• Statistical methods: All analyses were two-tailed with a minimum significance level of 5%. One way analysis of variance followed by Dunnett's test was used to analyze maternal and fetal data including body weights, food consumption, number of viable fetuses, implantation sites, and corpora lutea. Mann-Whitney U test was used to compare post-implantation loss, dead fetuses, and resorptions. Fetal sex ratios were analyzed using the Chi-Square test. Fisher's Exact test was used to analyze the incidence and number of fetal malformations and variations utilizing the darn (litter) as the experimental unit.
• Remarks field for Test Conditions:
- Age at study initiation: 3-5 kg
- Number of animals per dose per sex: 20 pregnant females
- Vehicle: 1% aqueous carboxymethyl cellulose
- Clinical observations: During the experimental period, all animals were observed daily for clinical signs of toxicity including physical or behavioral abnormalities. Mortality cheeks were performed twice daily, in the morning and afternoon. In addition, during the treatment period, the rabbits were observed between one-half hour and two hours after dosing for toxic effects.
- Body Weights: Individual maternal body weights were measured on gestation days 0, 6, 9, 12, 15, 19, 24 and 29. Body weight changes were calculated for the following gestation intervals: 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29.
- Mating procedures: Females were artificially inseminated. Semen was collected from New Zealand White rabbits obtained from the same source and supplier as females and evaluated for volume, motility, and concentration. The semen was diluted with 0.9% physiological saline and maintained in a water bath at 36 °C during the insemination procedure. Approximately 0.5 ml of the diluted semen was introduced into the female's vagina. Semen from one male was used to inseminate an equal number of females in each study group. Immediately following insemination, the females were administered human chronic gonadotropin, via the marginal ear vein. The day of insemination was considered day 0 of gestation.
- Parameters assessed during study: Individual food consumption was measured daily during gestation. Food consumption was calculated as grams/animal/day and grams/kilogram/day and reported for gestation days 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, 24-29, 6-19, 19-29 and 0-29.

- Organs examined at necropsy: Females that aborted during the study were sacrificed and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. Uterine contents were examined and the number of implants was recorded. The number of corpora lutea on each ovary was noted.
All surviving females were sacrificed on gestation day 29 and subjected to a morphological examination.
Cesarean Section Observations - The uterus was removed from the body, examined externally, weighed and then opened for an internal examination. The number of viable and nonviable fetuses, early and late resorptions was recorded beginning with the left distal uterine horn, noting the position of the cervix, and continuing with the right uterine horn. Corpora lutea were counted and recorded for each ovary. Uteri with no macroscopic evidence of implants were placed in 10% aqueous ammonium sulfide for detection of early embryolethality as described by Salewski.
Fetal Morphological Observations - Fetuses were examined for external, internal (visceral), and skeletal anomalies. Developmental malformations and variations were classified based upon the severity of the anatomical change(s) and the extent of their interference with organ and/or body functions.
Each fetus was examined for the occurrence of external abnormalities. The fetuses were weighed and tagged individually. The crown-rump length of late resorptions was measured and the tissues discarded.
Each fetus was dissected and examined using a technique similar to that described by Staples. During the procedure, the sex of the fetus was determined. The examination was performed under low power magnification.
Each fetus was eviscerated, skinned and fixed in 95% isopropyl alcohol. Following fixation, the fetuses were macerated in 1.5% aqueous potassium hydroxide solution, stained with Alizarin Red S, and cleared in 25% aqueous glycerin solution. Subsequent skeletal examinations were performed using substage lighting.

RESULTS

• NOAEL maternal toxicity: Study Director's: 50 mg/kg bw
• NOAEL developmental toxicity: Study Director's: 500 mg/kg bw
• Actual dose received by dose level by sex if available: Concentration analyses revealed average test article recoveries from the suspensions of sodium isocyanurate in methylcellulose ranged from 99.9% to 109.4%, indicating that the dosing solutions were accurately prepared.
• Fetal data: No treatment-related differences were noted in the Cesarean section parameters evaluated between the control, 50 and 200 mg/kg/day groups.
• Statistical results, as appropriate: N/A
• Remarks field for Results:
- Mortality and day of death: One female at the 500 mg/kg/day level and two females in the 50 mg/kg/day group aborted on gestation days 22, 24 and 26, respectively and were sacrificed and necropsied. All other females in the control and treatment groups survived to scheduled sacrifice on gestation day 29.
- Number pregnant per dose level: The pregnancy rate was 100% in the 50 mg/kg/day group, 90% in the control and 200 mg/kg/day groups, and 80% in the 500 mg/kg/day group.
- Number aborting: One female at the 500 mg/kg/day level and two females in the 50 mg/kg/day group aborted on gestation days 22, 24 and 26, respectively.
- Number of resorptions, early/late if available: see table below
- Number of implantations: see table below
- Pre and post implantation loss, if available: A statistically significant increase was noted in post implantation loss at the 500 mg/kg/day level resulting from a slight increase in the mean number of late resorptions. This increase was due primarily to one dam in the group with seven late resorptions. The mean post-implantation loss in the 500 mg/kg/day group (1.5) was within the range of historical control data (0.2-1.9). It should be noted that much variation was observed in the number of late resorptions between the groups (3, 9, 2, and 16 late resorptions in the control, low, mid, and high dosage groups, respectively). The study directors concluded that the lack of a dose-dependent trend and any evidence of fetal toxicity (e.g., reduced fetal weight) indicated that the increased post-implantation loss in the 500 mg/kg/day group was random rather than treatment-induced.
- Number of corpora lutea: There were no treatment-related or statistically significant differences in the mean number of corpora lutea in the test article groups when compared to the control group.
- Duration of Pregnancy: There were no treatment-related or statistically significant differences in the duration of pregnancy in the test article groups when compared to the vehicle control and untreated control groups. One female at the 500 mg/kg/day level and two females in the 50 mg/kg/day group aborted between gestation days 22 and 26. All other females in the control and treatment groups survived to scheduled sacrifice.
- Body weight:
Maternal - No statistically significant differences were noted in mean body weight and body weight gain between the control and

treatment groups. A slight reduction in body weight gain and slight body weight loss occurred at the 500 mg/kg/day level during the latter part of treatment (gestation days 12-15 and 15-19, respectively). At the 200 mg/kg/day level, slight body weight loss occurred during gestation days 15-19. These changes resulted in slightly reduced body weight gains for the entire treatment period (gestation days 6-19) at the 200 and 500 mg/kg/day levels. Following the completion of treatment (gestation days 19-24), body weight gain at the 500 mg/kg/day level was slightly higher than the control group.
Fetal - Mean fetal body weight was comparable between the control and treatment groups.
- Food/water consumption: Food consumption calculated as grams/animal/day and grams/kilogram/day was slightly reduced at the 200 and 500 mg/kg/day levels during gestation days 15-19. Food consumption was slightly reduced at the 50 and 200 mg/kg/day levels during gestation days 24-29. Since the reductions at the 50 and 200 mg/kg/day levels occurred following the completion of treatment and a similar reduction was not observed at the 500 mg/kg/day level, the study directors did not consider the changes to be related to treatment. At the 500 mg/kg/day level, food consumption was slightly increased following the completion of dosing (gestation days 24-29).
- Description, severity, time of onset and duration of clinical signs: No treatment-related clinical signs of toxicity were observed during the study. Incidental findings observed throughout the control and treatment groups included few feces, soft stools and hair loss.
- Gross pathology incidence and severity: In dams, no treatment-related gross abnormalities were observed at necropsy. Incidental findings observed in the control and treatment groups included, fluid contents in the thoracic cavity, dark red lungs, small spleen, distended oviducts, subcutaneous cyst, uterine cyst, gallbladder adhesion and gallbladder absent.
- Organ weight changes, particularly effects on total uterine weight:
No treatment-related differences were noted in gravid uterus weight between the control, 50, 200 and 500 mg/kg/day groups.
- Fetal data:
Litter size and weights: The number of litters per dose group were 18, 20, 18 and 16 for the 0, 50, 200, and 500 mg/kg/day dose groups, respectively. There were no treatment-related or statistically significant differences in the fetal body weights amongst the various groups.
Number viable (number alive and number dead): A slight decrease in the mean number of viable fetuses was noted at the 200 mg/kg/day dose level. However, since a concomitant decrease in mean post-implantation loss did not occur, the change was not considered to be biologically significant.
Sex ratio: A statistically significant difference was noted in the fetal sex ratio at the 50 mg/kg/day level. However, since a similar difference was not noted at the 200 and 500 mg/kg/day levels, this change was considered incidental and not related to treatment with the test article.
Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: No statistically significant differences were noted in fetal malformation data. The incidence and number of litters with malformations were generally similar throughout the control and treatment groups. The malformations were generally dissimilar in nature and included filamentous tail, microphthalmia, flexed paw, spinal bifida, iris bombe, unascended kidneys, lung cysts, hydrocephaly, and vertebral, skull and rib anomalies. With the exception of one fetus in the 200 mg/kg/day group, all fetuses with hydrocephaly were also observed to have domed head during external examination. The developmental malformations, with the exception of hydrocephaly, occurred in a very low incidence (1 or 2 fetuses per malformation). The hydrocephaly occurred in three fetuses from one litter in the control group, three fetuses in two litters at the 200 mg/kg/day level, and nine fetuses from two litters in the 500 mg/kg/day group. However, the number of litters with fetuses exhibiting hydrocephaly was not remarkably different between the groups (1, 0, 2, and 2 in the same groups). Hydrocephaly is not uncommon for this species and strain. Furthermore, there was no evidence of developmental toxicity in a teratology study of Monosodium Isocyanurate conducted previously in rabbits with identical dosage levels; post-implantation loss, fetal weight, and the incidence of malformations were similar in the control and treated groups. Thus, the study directors considered the increased incidence of hydrocephaly in the present study as random and not treatment related. The incidence and number of litters with developmental variations was comparable between the control, 50, 200 and 500 mg/kg/day groups.

Summary of Fetal Observations - Malformations

		Fetuses				Litters			
	Group	1	2	3	4	1	2	3	4
	Level (mg/kg/day)	0	50	200	500	0	50	200	500
Number examined externally		128	126	106	82	18	18	17	15
Filamentous Tail		0	0	1	0	0	0	1	0
Microphthalmia		0	0	0	1	0	0	0	1
Flexed Paw		0	0	0	2	0	0	0	2
Spina Bifida		0	1	0	0	0	1	0	0
Number Examined Viscerally		128	126	106	82	18	18	17	15
Iris bombe		2	1	0	1	2	1	0	1
Unascended Kidney(s)		0	0	1	0	0	0	1	0
Lung Cysts		0	1	0	0	0	1	0	0
Hydrocephaly		3	0	3	9	1	0	2	2
Number Examined Skeletally		128	126	106	82	18	18	17	15
Vertebral Anomaly with/without rib anomaly		0	0	1	0	0	0	1	0
Skull anomaly		1	0	1	1	1	0	1	1
Rib anomaly		0	0	0	1	0	0	0	1
Total Malformations									
Number with External Malformations		0	1	1	3	0	1	1	2
Number with Soft Tissue Malformations		5	2	4	10	3	2	3	3
Number with Skeletal Malformations		1	0	2	2	1	0	2	2
Total Number of Malformations		6	3	6	12	4	3	4	5

Summary of Fetal Observations - Variations

		Fetuses				Litters			
	Group	1	2	3	4	1	2	3	4
	Level (mg/kg/day)	0	50	200	500	0	50	200	500
Number examined externally		128	126	106	82	18	18	17	15
Number with Findings		0	0	0	0	0	0	0	0
Number Examined Viscerally		128	126	106	82	18	18	17	15
Major Blood Vessel Variation		4	3	5	2	3	3	5	2
Retrocaval Ureter		1	3	0	5	1	2	0	2
Number Examined Skeletally		128	126	106	82	18	18	17	15
27 presacral vertebrae		24	17	6	9	12	9	6	6
13th full rib(s)		72	60	33	46	16	15	14	13
Accessory skull bone(s)		5	0	3	4	4	0	3	3
13th rudimentary rib(s)		28	32	26	13	11	15	13	6
25 presacral vertebrae		0	0	2	0	0	0	2	0
Sternebra(e) malaligned (slight or moderate)		12	8	10	6	1	5	1	5
Hyoid arch(es) bent		1	6	1	6	1	5	1	5
Sternebra #5 and/or #6 unossified		2	6	4	0	1	3	2	0
Costal cartilage malaligned		1	0	0	0	1	0	0	0
Sternebrae with thread-like attachment		1	0	2	1	1	0	1	1
Sternebra(e) #1, #2, #3 and/or #4 unossified		0	0	1	0	0	0	1	0

Summary of Cesarean Section Data

Group		1	2	3	4
Group Level		0 mg/kg/day	50 mg/kg/day	200 mg/kg/day	500 mg/kg/day
Females Gravid		18	18	18	15
Corpora Lutea	Total	200	206	190	172
	Mean	11.1	11.4	10.6	11.5
	S.D.	2.5	2.7	2.9	2.2
Implantation Sites	Total	138	138	120	105
	Mean	7.7	7.7	6.7	7.0
	S.D.	3.0	2.2	2.2	2.6
Pre-Implantation Loss	Total	62	68	70	67
	Mean	3.4	3.8	3.9	4.5
	S.D.	3.5	2.9	2.4	2.9
Viable Fetuses	Total	128	126	106	82
	Mean	7.1	7.0	5.9	5.5
	S.D.	2.9	2.1	2.6	2.7
Dead Fetuses	Total	0	0	0	1
	Mean	0.0	0.0	0.0	0.1
	S.D.	0.0	0.0	0.0	0.3
Late Resorptions	Total	3	9	2	16
	Mean	0.2	0.5	0.1	1.1
	S.D.	0.4	0.9	0.3	2.0
Early Resorptions	Total	7	3	12	6
	Mean	0.4	0.2	0.7	0.4
	S.D.	1.0	0.7	1.2	0.6
Post-Implantation Loss	Total	10	12	14	23
	Mean	0.6	0.7	0.8	1.5*
	S.D.	1.2	1.1	1.4	2.1
Sex M/F	Total	62 66	79 47	58 48	47 35
	Mean	3.4 3.7	4.4 2.6*	3.2 2.7	3.1 2.3
	S.D.	1.6 2.0	1.7 1.8	2.0 1.4	1.6 2.0
Gravid Uterus Weight (G)	Mean	452.8	486.4	372.9	406.3
	S.D.	130.1	110.0	142.6	172.6
	N	18	18	18	15
Fetal Weight (G)	Mean	45.8	46.9	44.9	46.8
	S.D.	6.2	6.1	5.7	5.3

Summary of Survival and Pregnancy

Group	1		2		3		4	
Group Level	0 mg/kg/day		50 mg/kg/day		200 mg/kg/day		500 mg/kg/day	
	No.	%	No.	%	No.	%	No.	%
Females on Study	20		20		20		20	
Females that Aborted	0	0.0	2	10.0	0	0.0	1	5.0
Females Examined At								
- Scheduled Necropsy	20	100.0	18	90.0	20	100.0	19	95.0
- Nongravid	2	10.0	0	0.0	2	10.0	4	21.1
- Gravid	18	90.0	18	100.0	18	90.0	15	78.9
- with resorptions only	0	0.0	0	0.0	1	5.6	0	0.0
- with viable fetuses	18	100.0	18	100.0	17	94.4	15	100.0
Total Females Gravid	18	90.0	20	100.0	18	90.0	16	80.0

CONCLUSIONS

Study Director's Comments: "Oral administration of Monosodium Isocyanurate to pregnant rabbits during the major period of organogenesis produced maternal toxicity at levels of 200 and 500 mg/kg/day. There was no evidence of developmental toxicity in any of the treated groups. Therefore, a dosage level of 50 mg/kg/day was considered a NOEL for maternal toxicity. A level of 500 mg/kg/day was considered a NOEL for developmental toxicity."

DATA QUALITY

• Reliability: Klimisch Code 1a (Reliable without restriction; Meets OECD 414 "Teratogenicity" guideline)

REFERENCES (Free text):

"Teratology Study in Rabbits with Monosodium Isocyanurate," Springborn Laboratories Inc., 3222.1, December 4, 1990; MRID 42054101.

19a

Toxicokinetics (Metabolism)

TEST SUBSTANCE

• Identity: Sodium cyanurate monohydrate (s-triazinetriol, monosodium salt); CAS No. 2624-17-1
• Remarks field for Test Substance:
- Unlabeled sodium cyanurate monohydrate: 99.5% pure
- ^{14}C-labelled sodium cyanurate monohydrate: 9.8 mCi/mmol, >99% radio chemical purity
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: OECD Method 417
• GLP (Y/N): NA
• Year (study performed): 1981-1982
• Species/Strain: Rat/Sprague-Dawley
• Sex: Male/female
• Age: 8-10 weeks old
• Doses:
- Oral: 5 or 500 mg/kg (single dose)
- Oral: 5 mg/kg (14 daily doses with unlabeled sodium cyanurate followed by one dose with radiolabelled sodium cyanurate)
- Intravenous: 5 mg/kg (single dose)
• Number of animals per dose: 5/sex/dose
• Remarks field for Test Conditions:
- Analytical method for radiolabel: Radioactivity was determined in the samples by scintillation counting following appropriate extraction and concentration procedures.
- Analytical method for test substance: Urine samples were extracted, evaporated to dryness and reconsolidated for liquid chromatographic analysis.

RESULTS

• Elimination half-life at 5 mg/kg: 30 to 60 minutes
• Elimination half-life at 500 mg/kg: 2.5 hours
• Remarks field for Results:
- At the low dose the test substance was completely absorbed and largely eliminated in urine. At the higher dose the substance was incompletely absorbed and a larger percentage eliminated in faeces. Radioactivity remaining in tissues was below the level of detection (0.1-1.0 µg/g) for most tissues. No radioactivity was exhaled as $^{14}CO_2$.
- Only parent compound was found in urine, indicating that metabolism of sodium cyanurate did not occur or was minimal.

CONCLUSIONS

Study conclusions: Sodium cyanurate is well absorbed following administration at 5 mg/kg to rats, but absorption was saturable at the 500 mg/kg dose level. Metabolism of the compound, if it occurs at all, is negligible. The majority of the compound is rapidly eliminated in urine. A small fraction of the dose is more slowly eliminated. This fraction is insufficient to result in appreciable bioaccumulation on a daily dose schedule. There are no major changes in the disposition or metabolism of sodium cyanurate following its repeated administration.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guidline study)
• Remarks field for Data Reliability: The EPA Data Evaluation Report classifies this study as “Guideline”.

REFERENCES (Free Text):

A. D. Little, Inc., “Disposition and Metabolism of 14C-Labeled Sodium Cyanurate in Rat”, Report to Isocyanurate Industry Ad Hoc Committee, Project No. C-86329, 1982.

EPA Data Evaluation Report, August 4, 1983, MRID 131014.

Summary in: Barbee, S. J., Casieri, T., Hammond, B. G, Inoue, T., Ishida, N., Wheeler, A. G., Chadwick, M., Hayes, D., Macauley, J., and McComish, A. “Metabolism and Disposition of Sodium Cyanurate”, Toxicologist 3: 80 (1983).

19b

Toxicokinetics (Metabolism)

TEST SUBSTANCE

• Identity: Sodium cyanurate monohydrate; CAS No. 2624-17-1
• Remarks field for Test Substance:
- Unlabeled sodium cyanurate monohydrate: 99.5% pure
- ^{14}C-labelled sodium cyanurate monohydrate: 9.8 mCi/mmol, >99% radio chemical purity
- Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: OECD Method 417
• GLP (Y/N): NA
• Year (study performed): 1981-1982
• Species/Strain: Dog/Beagle
• Sex: Male/female
• Age: 5.9-8.5 months old
• Doses:
- Oral: 5 or 500 mg/kg (single dose)
- Oral: 5 mg/kg (14 daily doses with unlabeled sodium cyanurate followed by one dose with radiolabelled sodium cyanurate)
- Intravenous: 5 mg/kg (single dose)
• Number of animals per dose: 4/sex/dose for single dose groups, 2/sex/dose for 15 day dose groups
• Remarks field for Test Conditions:
- Analytical method for radiolabel: Radioactivity was determined in the samples by scintillation counting following appropriate extraction and concentration procedures.
- Analytical method for test substance: Urine and fecal samples were analyzed quantitatively by high performace liquid chromatography and thin layer chromatography to identify metabolites.

RESULTS

• Elimination half-life at 5 mg/kg: 1.5-2.0 hours
• Elimination half-life at 500 mg/kg: 2 hours
• Remarks field for Results:
- At the low dose the test substance was completely absorbed and largely eliminated in urine. At the higher dose the substance was incompletely absorbed and the remainder was excreted in the fecies. At sacrifice, radioactivity remaining in all tissue samples was below the level of detection.
- Total recovery of cyanurate was similar for all dose regimens and ranged from 81 to 101%. Cyanurate was excreted unchanged in the urine under all dose regimens, indicating that metabolism of sodium cyanurate did not occur.

CONCLUSIONS

Study conclusions: Sodium cyanurate is well absorbed following intravenous and oral administration at 5 mg/kg to dogs, but absorption from the gastrointestinal tract is saturable at high doses. Metabolism of the compound, if it occurs at all, is negligible. The compound is relatively rapidly eliminated in the urine. There is no evidence that bioaccumulation of the compound would occur on a daily dose schedule at 5 mg/kg. There are no major changes in the disposition or metabolism of sodium cyanurate following its repeated administration.

DATA QUALITY

• Reliability: Klimisch Code 1b (Reliable without restriction; Comparable to guidline study)

• Remarks field for Data Reliability: The EPA Data Evaluation Report classifies this study as “Minimum”.

REFERENCES (Free Text):

A. D. Little, Inc., “Disposition and Metabolism of 14C-Labeled Sodium Cyanurate in Dog”, Report to Isocyanurate Industry Ad Hoc Committee, Project No. C-86329, 1982.

EPA Data Evaluation Report, August 4, 1983, MRID 128287.

Summary in: Barbee, S. J., Casieri, T., Hammond, B. G, Inoue, T., Ishida, N., Wheeler, A. G., Chadwick, M., Hayes, D., Macauley, J., and McComish, A. “Metabolism and Disposition of Sodium Cyanurate in the Dog”, Toxicologist 4: 92 (1984).

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Toxicokinetics (Metabolism)

TEST SUBSTANCE

• Identity: Cyanuric Acid; CAS No. 108-80-5
Remarks field for Test Substance: Both s-triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-trichloro- and s-triazine-2,4,6(1H,3H,5H)-trione, 1,3-dichloro-, sodium salt are unstable in the body (particularly the stomach) because the available chlorine is rapidly reduced. Cyanuric acid (or its monosodium salt) is the degradation product from both products.

METHOD

• Method/guideline followed: Swimmers completely voided prior to swimming for 120 minutes and thereafter as necessary. Urine samples were analyzed quantitatively for cyanuric acid.
• GLP (Y/N): No
• Year (study performed): 1981-1982
• Species/Strain: Human
• Sex: Male/female
• Age/Weight: 9-17 years old / 29-74 kg
• Number of animals: 1 male, 4 female
Remarks field for Test Conditions:
- Analytical method for test substance: Cyanuric acid was extracted from urine samples by a multistep process and then analyzed quantitatively by high performace liquid chromatography. Sensitivity was 0.5 mg/L cyanuric acid in urine. Recovery of 10 mg/L cyanuric acid spikes in urine free of cyanuric acid gave an average recovery of >99%.
- The elimination kinetics were analyzed by weighted nonlinear least-squares regression.

RESULTS

• Elimination half-life: 2.2-3.5 hours
• Remarks field for Results:
- The minimum compartmental model found to be statistically consistent with the urine excretion data for cyanuric acid was the one-compartment open model with first order input and elimination.
- After soaking in a pool for 120 minutes, the subsequent cumulative excretion of cyanuric acid was 0.03-2.8 mg and was equivalent to 3.0-3.6 mL of pool water.
- Two volunteers drank solutions of known cyanuric acid concentration. Analysis of their 24 hour cumulative urine excretion indicated greater than 98% recovery of orally ingested cyanuric acid.

CONCLUSIONS

Study conclusions: Cyanuric acid is excreted rapidly and nearly quantitatively after ingestion. The kinetics of elimination conform to a one-component open model. The elimination half-life is approximately three hours.

DATA QUALITY

• Remarks field for Data Reliability: This published scientific paper provides useful human test data.

REFERENCES (Free Text):

Allen, M. A., Briggle, T. V., and Pfaffenberger, C. D., “Absorption and Excretion of Cyanuric Acid in Long-Distance Swimmers”, Drug Metabolism Reviews, 13(3) 499-516 (1982).