Cyanazine in Drinking-water

Background document for development of WHO Guidelines for Drinking-water Quality

Preface

One of the primary goals of WHO and its member states is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose regulations, and to make recommendations with respect to international health matters ....”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as International Standards for Drinking-Water. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO Guidelines for drinking-water quality (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A “final task force” meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health
Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues, and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.
Acknowledgements

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The work of the following coordinators was crucial in the development of this document and others in the Addendum:

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Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

The efforts of all who helped in the preparation and finalization of this document, including those who drafted and peer reviewed drafts, are gratefully acknowledged.

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GENERAL DESCRIPTION

Identity
CAS no: 21725-46-2
Molecular formula: C₉H₁₃ClN₆

Cyanazine is a member of the triazine family of herbicides. The IUPAC name for cyanazine is 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-yl)amino-2-methyl propionitrile.

Physicochemical properties [Source: CHEMLAB's Chemical Information System. Bethesda, MD, CIS, Inc. (1985)]

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>White solid at 25°C</td>
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<tr>
<td>Melting point</td>
<td>167.5–169°C</td>
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<tr>
<td>Density</td>
<td>0.35 (fluffed), 0.45 (packed) g/cm³</td>
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<tr>
<td>Vapour pressure</td>
<td>2.1 × 10⁻⁷ to 10.0 × 10⁻⁷ Pa at 20°C</td>
</tr>
<tr>
<td>Water solubility</td>
<td>171 mg/litre at 25°C</td>
</tr>
<tr>
<td>Log octanol–water partition coefficient</td>
<td>2.24</td>
</tr>
</tbody>
</table>

Major uses

Cyanazine is used as a pre- and post-emergence herbicide for the control of annual grasses and broadleaf weeds (Meister, 1983).

Environmental fate

Cyanazine can be degraded in soil and water by microorganisms by N-dealkylation and hydrolysis. Cyanazine readily leaches from soil. In laboratory tests, cyanazine (5–10 mg/litre) had a half-life of 2–14 weeks in four kinds of soils at 22°C (Osgerby et al., 1968). Four degradation products can be identified for cyanazine — the amide, two acids, and the amine. Aerobically and anaerobically aged cyanazine residues, primarily the amide degradation product, are intermediately mobile to mobile on sandy clay loam soil (Eadsforth, 1984). The amide degradation product is predominant in the leachate from sandy soil; the acid degradation products predominate in leachate from loamy sand and sandy loam soils. Unaltered cyanazine can also be identified in soil leachate.

ANALYTICAL METHODS

Cyanazine in water samples can be analysed using a high-performance liquid chromatographic (HPLC) method (Method #4; US EPA, 1986b). In this method, 1 litre of sample is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 ml or less. HPLC is used to separate compounds, and measurement is conducted with an ultraviolet detector. Using this method, the estimated detection limit for cyanazine is 0.3 µg/litre.
ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Water

In the USA, cyanazine was detected in surface water and groundwater at maximum concentrations of 1300 and 3500 µg/litre, respectively. Cyanazine was identified in drinking-water in New Orleans, Louisiana, at concentrations ranging from 0.01 to 0.35 µg/litre [Source: STORET water quality file. Washington, DC, US Environmental Protection Agency, Office of Water (data file search conducted in May 1988)]. Cyanazine was also found in surface water in Ohio River basins (Datta, 1984) and in groundwater in Iowa and Pennsylvania; typical detectable concentrations ranged from 0.1 to 1.0 µg/litre (Cohen et al., 1986). Monitoring data in a reservoir on the Des Moines River in Iowa from September 1977 through November 1978 indicated that agricultural runoff (from corn and soybean) was a major source of cyanazine in the river: levels of 71–457 and 2–151 ng/litre were detected during the active months of May through August and during September through December, respectively; cyanazine was not detected from January through April (NAS, 1977; US EPA, 1984a).

Cyanazine was detected (detection limits 0.025–1 µg/litre) in 9 of 1128 samples of municipal and private water supplies in three Canadian provinces between 1978 and 1986; concentrations ranged from <0.1 to 4.0 µg/litre (Hiebsch, 1988). It was also detected (detection limit 0.02 µg/litre) in 45 of 440 surface water samples from three Ontario, Canada, river basins surveyed between 1981 and 1985; mean detectable concentrations were 0.8, 0.1, and 1.5 µg/litre in the three basins (Frank & Logan, 1988).

Cyanazine has been found in groundwater in the Netherlands at concentrations above 0.1 µg/litre. It was not detected in surface water used as a source for drinking-water (Council of Europe, 1993).

Food

Data on levels of cyanazine residues in food are not available. It is expected that the intake of cyanazine from food is very low, because no residues of cyanazine or its degradation products have been detected in crops following application (Department of National Health and Welfare, 1986).

Estimated total exposure and relative contribution of drinking-water

Based on Canadian monitoring data and the data from the Netherlands, the daily intake of cyanazine from drinking-water can be estimated to fall in the range of 0.2–3 µg. As there is no information available on concentrations of cyanazine in food or air, the relative contribution of drinking-water to total daily intake cannot be estimated.

KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Cyanazine is rapidly absorbed from the gastrointestinal tract of rats, dogs, and cows (Shell Chemical Company, 1969; Hutson et al., 1970; Crayford & Hutson, 1972). Measurements of urinary, faecal, and biliary excretion indicated that 80–88% of the administered dose was eliminated within 4 days in rats and dogs and within 21 days in cows. In rats, elimination in urine was almost equal to elimination in faeces. In dogs and cows, approximately one-half of the dose was eliminated in the urine, and about one-third was eliminated in the faeces. In cows, the amount of residues excreted daily was constant throughout the study period. Cyanazine was also detected in cows' milk.
The degradation of cyanazine follows metabolic pathways involving dealkylation and conjugation with glutathione. Seven metabolites were identified in rats: five in urine and two in faeces. N-De-ethylation was the major route of degradation of cyanazine; 47% of the leaving ethyl group was eliminated by exhalation (Shell Chemical Company, 1969; Crayford & Hutson, 1972).

Crayford et al. (1970) studied the metabolism of two of the major plant metabolites of cyanazine — 2-hydroxy-4-ethylamino-6-(1-carboxy-1-methylamino)-s-triazine and 2-hydroxy-4-amino-6-(1-carboxy-1-methylethylamino)-s-triazine — in rats. Approximately 91% of the first compound and 84% of the second compound were recovered unchanged from urine and faeces.

EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

Acute exposure

WHO (1996) has classified cyanazine as "moderately hazardous." Acute oral LD₅₀s in rats ranged from 149 to 835 mg/kg of body weight (SRI, 1967; Young & Adamik, 1979b; Meister, 1983; NIOSH, 1987). In these studies, the percentage of active ingredient (a.i.) in the tested products was not clearly identified. However, studies with technical cyanazine (97% a.i.) in rats, mice, and rabbits showed LD₅₀s of 182, 380, and 141 mg/kg of body weight, respectively (Walker et al., 1974).

Cyanazine caused mild eye irritation at 100 mg (Young & Adamik, 1979a) and slight skin irritation at 2000 mg (Young & Adamik, 1979c) in rabbits. A skin sensitization test in guinea-pigs was negative (Walker et al., 1974; Young & Adamik, 1979d).

The acute dermal LD₅₀ in rabbits treated with technical cyanazine (purity unspecified) was >2000 mg/kg of body weight (SRI, 1967; Young & Adamik, 1979c); in rats, the LD₅₀ was >1200 mg/kg of body weight (97% a.i.) (Walker et al., 1974).

Short-term exposure

In a 13-week feeding study in mice at levels of 0, 10, 50, 500, 1000, or 1500 mg/kg of feed (equivalent to 0, 1.3, 6.5, 65, 130, and 195 mg/kg of body weight per day; US EPA, 1986a), reduction in body-weight gain and statistically significant increases in relative liver weights were observed in both sexes at 65 mg/kg of body weight per day and above. The NOAEL was 6.5 mg/kg of body weight per day (Fish et al., 1979).

In a study by Walker et al. (1968), groups of 10 female CFE rats were treated by gavage with single oral doses of 1, 5, or 25 mg/kg of body weight of a wettable powder formulation (75% a.i.); the control group received water. No diuretic effects were produced; however, serum protein and potassium concentrations increased at the high dose, and serum osmolality increased at 5 mg/kg of body weight. The NOAEL appeared to be 1 mg/kg of body weight; however, this study did not provide enough information to allow the presence or absence of more significant effects at this dosage level to be determined.

In a 4-week oral toxicity study, groups of 10 male and 10 female CFE rats received diets containing cyanazine (75% or 97% a.i.) at 1, 10, or 100 mg/kg of feed (equivalent to 0.05, 0.5, and 5 mg/kg of body weight per day; US EPA, 1986a). A control group of 20 animals per sex was used. Reductions in body weight and food intake were noted at the high dose level. The LOAEL appeared to be 0.05 mg/kg of body weight per day based on kidney function tests, although additional information was not available to determine if any other significant adverse effects were noted at this level.
In 13-week feeding studies in rats given cyanazine doses of 0, 1, 1.5, 3, 6, 12, 25, 50, or 100 mg/kg of feed (equivalent to 0, 0.05, 0.075, 0.15, 0.30, 0.60, 1.25, 2.5, and 5 mg/kg of body weight per day), decreased body-weight gain was noted in males at 0.075 mg/kg of body weight per day and above and in females at 2.5 mg/kg of body weight per day and above. The NOAEL ranged from 0.05 to 1.25 mg/kg of body weight per day (Walker & Stevenson, 1968b).

In a 13-week oral study, beagle dogs were given cyanazine in gelatin capsules at 0, 1.5, 5, or 15 mg/kg of body weight per day. Emesis was noted within the first hour of dosing in all of the high-dose males. Reduced body-weight gain was also noted in the high-dose group during the second half of the study period, as well as increased kidney and liver weights in the females of this group. The NOAEL was 5 mg/kg of body weight per day (Walker & Stevenson, 1968a; Walker et al., 1974).

**Long-term exposure**

In a 2-year study in mice given cyanazine (96.4% pure) at doses of 0, 10, 25, 250, or 1000 mg/kg of feed (equivalent to 0, 1.3, 3.3, 33, and 130 mg/kg of body weight per day), survival ranged from 46 to 58% in males and from 38 to 50% in females; survival was slightly reduced in females but not in males at the two highest doses. Mean body-weight gains for the entire study were reduced 4% from controls in both sexes at 25 mg/kg of feed and 18% and 25% in males and females, respectively, receiving 250 mg/kg of feed. At 1000 mg/kg, weight gains were reduced 25% and 32% in males and females, respectively; the MTD was exceeded at this dose. Non-neoplastic histological findings at 250 mg/kg of feed and above included increased incidences of parenchymal atrophy of the liver in females, kidney toxicity, and skin ulceration. Based on decreased weight gains and histological changes, the NOAEL for systemic toxicity was 25 mg/kg of feed (3.3 mg/kg of body weight per day) (Gellatly, 1981).

In a 2-year study, Sprague-Dawley rats (50 per sex per dose) were exposed to cyanazine (96% pure) at 0, 1, 5, 25, or 50 mg/kg of feed (Bogdanfy, 1990). An additional 10 animals per sex per dose were used as satellite animals for biochemical testing, then sacrificed after 12 months. The mean daily intakes were 0, 0.040, 0.198, 0.985, or 2.06 mg/kg of body weight for the males and 0, 0.053, 0.259, 1.37, or 2.81 mg/kg of body weight for the females. No adverse effects on survival were observed. A significant increase in the incidence of hyperactivity was observed in males receiving 25 mg/kg of feed (40%) and 50 mg/kg of feed (58%) compared with controls (20%), but no hyperactivity was observed in females. The incidence of palpable masses was significantly (p < 0.05) increased for females at the highest dose (51% vs 38% for controls), and the median time to first observed mass was decreased (343 days) compared with controls (406 days). Mean body weights and body-weight gains were significantly depressed during the first year of the study in males receiving 50 mg/kg of feed (up to 18%) and females receiving 25 or 50 mg/kg of feed (about 20%). A significant (p < 0.05) increase in the incidence of extramedullary haematopoiesis of the spleen was observed in males in the highest dose group (56% vs 39% for controls), and there was a significant trend (p < 0.02) for granulocytic hyperplasia of the bone marrow (p = 0.05). In highest-dose females, sciatic nerve demyelination was increased (18% vs 8% for controls), with a positive dose trend (p = 0.013). The NOAEL was 5 mg/kg of feed (0.198 mg/kg of body weight per day for males; 0.259 mg/kg of body weight per day for females) based on hyperactivity in male rats and decreased body-weight gain in females.

In a 2-year study in beagle dogs with technical cyanazine (97% a.i. in gelatin capsules) at dose levels of 0, 0.625, 1.25, or 5 mg/kg of body weight per day, frequent emesis was noted within 1 hour of dosing in the high-dose group; this effect was associated with reduction of growth rate and serum protein. The NOAEL appeared to be 1.25 mg/kg of body weight per day (Walker et al., 1970). Because of inadequate histopathology and data reporting in this study, a 1-year feeding study in beagle dogs was later performed by Dickie (1986) at
cyanazine (98% pure) levels of 0, 10, 25, 100, or 200 mg/kg of feed (equal to 0, 0.27, 0.68, 3.20, and 6.11 mg/kg of body weight per day for males and 0, 0.28, 0.72, 3.02, and 6.39 mg/kg of body weight per day for females). No systemic toxicity was noted at 10 or 25 mg/kg of feed. At 100 and 200 mg/kg of feed, dose-related decreases in body weight and body-weight gains were observed, platelet counts were occasionally elevated (non-significantly), liver-to-body-weight ratios were slightly increased, and kidney-to-body-weight ratios were slightly increased (p < 0.05) in females. Decreased serum levels of total protein albumin and calcium were consistently noted in both sexes at 200 mg/kg of feed. The average NOAEL for systemic toxicity in males and females was 0.7 mg/kg of body weight per day.

Reproductive and developmental toxicity

No significant effects on reproductive parameters were found in a three-generation study in Long-Evans rats using technical cyanazine (unknown percentage a.i.) at levels of 0, 3, 9, 27, or 81 mg/kg of feed (equivalent to 0, 0.15, 0.45, 1.35, and 4.05 mg/kg of body weight per day; US EPA, 1986a). The NOAEL in this study appeared to be 1.35 mg/kg of body weight per day based on reduced body-weight gain in parental animals and increased brain weight and decreased relative kidney weight in F3b female weanlings (Eisenlord et al., 1969).

In a two-generation reproductive study in Sprague-Dawley rats, cyanazine (100% a.i.) was administered at 0, 25, 75, 150, or 250 mg/kg of feed (equal to intake in dams of 0, 1.8, 5.3, 11.1, and 18.5 mg/kg of body weight per day; intake changed during lactation to 0, 3.8, 11.2, 23.0, or 37.1 mg/kg body weight per day). There were no compound-related effects on the number of females producing litters or on litter size. Based on decreased pup viability and decreased mean pup body weight during lactation, the NOAEL for reproductive toxicity was 3.8 mg/kg of body weight per day. Dose-related parental toxicity was observed at the lowest dose tested, as body weights of F0 and F1 adults of both sexes decreased throughout the study period (WIL Research Laboratories, 1987; Dapson, 1990).

In a study by Lu et al. (1981, 1982), Fischer 344 rats (30 dams per group) were administered cyanazine (98.5% a.i.) by gavage (suspended in a 0.2% Methocel emulsion) at dose levels of 0, 1.0, 2.5, 10, or 25 mg/kg of body weight per day on gestation days 6–15. Maternal body-weight reductions were noted at 10 and 25 mg/kg of body weight per day. Diaphragmatic hernia associated with liver protrusion, microphthalmia, and anophthalmia were observed at 25 mg/kg of body weight per day.

Lochry et al. (1985) repeated the above study, administering cyanazine (98% a.i.) to dams (70 per dose group) by gavage in an aqueous suspension of 0.25% (w/v) methyl cellulose at dose levels of 0, 5, 25, or 75 mg/kg of body weight per day on days 6–15 of gestation. Maternal body-weight reductions were noted in all dosage groups and appeared to be partly associated with lower food intake during the dosing period. Alteration in skeletal ossification sites was observed in the fetuses at all dose levels. Teratogenic effects — anophthalmia/microphthalmia, dilated brain ventricles and cleft palate in the fetuses, and abnormalities of the diaphragm (associated with liver protrusion) in pups sacrificed at time of weaning — were demonstrated at 25 and 75 mg/kg of body weight per day. Maternal and developmental toxicity were observed at 5 mg/kg of body weight per day (lowest dose tested), and the NOAEL for teratogenic effects was 5 mg/kg of body weight per day (Bui, 1985).

In an additional study in Sprague-Dawley rats, oral administration of cyanazine at 30 mg/kg of body weight per day (the highest dose tested) resulted in maternal body-weight reductions and increased incidence of piloerection; no developmental toxicity was observed. The maternal systemic NOAEL was 3 mg/kg of body weight per day (Shell Chemical Company, 1983).
New Zealand white rabbits (22 dams per dose) were orally dosed with cyanazine (98% a.i.) in gelatin capsules at levels of 0, 1, 2, or 4 mg/kg of body weight per day on gestation days 6–18. At 2 and 4 mg/kg of body weight per day, maternal toxic effects included anorexia, weight loss, death, and abortion; alterations in skeletal ossification sites, decreased litter size, and increased post-implantation loss were also observed. Malformations at 4 mg/kg of body weight per day included anophthalmia/microphthalmia, dilated brain ventricles, domed cranium, and thoracoschisis; however, these responses were observed at levels in excess of maternal toxicity. The NOAEL for both maternal and developmental toxicity was 1 mg/kg of body weight per day (Shell Toxicology Laboratory [Tunstall], 1982).

**Mutagenicity and related end-points**

The mutagenicity studies for cyanazine provide equivocal evidence for genotoxicity. Cyanazine induced dose-related forward mutation with and without metabolic activation in the mouse lymphoma L5178Y/TK cell gene mutation assay (Jannasch & Sawin, 1986). Cyanazine was positive for in vitro unscheduled DNA synthesis in repeat assays using rat primary hepatocytes (Vincent, 1987). Cyanazine was negative in an in vivo unscheduled DNA synthesis assay in rat spermatocytes to examine possible interaction with germ cells, a Salmonella assay, a Chinese hamster ovary/hprt gene mutation assay, and an in vitro human lymphocyte/aberrations assay (Stahl, 1987). Cyanazine was found to be negative in a Salmonella assay with rodent metabolic activation, but positive with a plant-derived activation system (Plewa et al., 1984).

**Carcinogenicity**

Cyanazine was not carcinogenic in mice (Gellatly, 1981; Shell Chemical Company, 1981). However, in Sprague-Dawley rats, dietary administration of 0, 1, 5, 25, or 50 mg/kg of feed for 2 years caused statistically significant increases in malignant mammary gland tumours (adenocarcinoma and carcinosarcoma) in females at incidences of 5/58 (8%), 7/61 (11%), 12/60 (20%), 20/62 (32%), and 15/62 (24%), respectively, with a significant positive trend (p = 0.0049). Statistical comparison excluded rats that died before week 48 when the first tumour occurred. The incidences of these tumours in dosed rats were outside the historical control data.

Atrazine, which has a chemical structure similar to that of cyanazine, has been found to increase the incidence of mammary tumours in rats and has been classified by IARC (1991) in Group 2B (agent is possibly carcinogenic to humans). The hypothesis that a hormonal mechanism of action may be involved in the manifestation of mammary gland tumours in rats upon exposure to triazine herbicides is currently under investigation.

**EFFECTS ON HUMANS**

No information was found in the available literature on the health effects of cyanazine in humans.

**GUIDELINE VALUE**

On the basis of the available mutagenicity data on cyanazine, evidence for genotoxicity is equivocal. Cyanazine causes mammary gland tumours in Sprague-Dawley rats but not in mice. The mechanism of mammary gland tumour development in Sprague-Dawley rats is currently under investigation, and a hormonal mechanism of action may be involved. Cyanazine is also teratogenic in Fischer 344 rats at dose levels of 25 mg/kg of body weight per day and higher.
Based on a 2-year toxicity/carcinogenicity study in rats (Bogdanfy, 1990), a NOAEL of 0.198 mg/kg of body weight per day has been identified, based on hyperactivity in male rats. By applying an uncertainty factor of 1000 (100 for inter- and intraspecies variation and 10 for limited evidence of carcinogenicity), a TDI of 0.198 µg/kg of body weight can be calculated. With an allocation of 10% of the TDI to drinking-water and assuming a 60-kg adult consuming 2 litres of drinking-water per day, the guideline value is 0.6 µg/litre (rounded figure).

REFERENCES


