Scand J Work Environ Health 1998;24 suppl 2:5—9

5226_V

Toxicologic profile of acrylonitrile

by Ruud A Woutersen, PhD[1]

Woutersen RA. Toxicologic profile of acrylonitrile. *Scand J Work Environ Health* 1998;24 suppl 2:5—9.

Acrylonitrile is a monomer used extensively as a raw material in the manufacturing of acrylic fibers, plastics, synthetic rubbers, and acrylamide. It has been classified as a probable human carcinogen according to the results of numerous chronic rat bioassays. The present report summarizes the toxicity data on acrylonitrile and reviews available data concerning the mechanism (genetic versus epigenetic) by which acrylonitrile is carcinogenic in rats. From the evaluation of the relevant toxicity data, it can be concluded that acrylonitrile is indeed carcinogenic to rats after either oral or inhalational exposure. However, information on other mammalian species is lacking, and, moreover, the exact mechanism of the carcinogenic process is unclear. Therefore, it is recommended to conduct an additional long-term inhalation carcinogenicity study with acrylonitrile in mice, as well as studies into the mechanism by which acrylonitrile induces (brain) tumors in rats (genetic versus epigenetic).

Key terms carcinogenicity, inhalation, mechanism, oral, toxicity.

Acrylonitrile is a monomer used extensively as a raw material in the manufacture of acrylic fibers, plastics, synthetic rubbers, and acrylamide. Apart from occupational exposure, concerns have been raised pertaining to potential exposure of the general public to acrylonitrile from food packaging and other consumer products. Numerous chronic rat bioassays have been conducted by various routes to provide a better understanding of the potential of acrylonitrile to cause cancer in humans. In the United States, the Environmental Protection Agency (1, 2) classified acrylonitrile as a group B1 chemical (probable human carcinogen) based on an increase in lung cancer among exposed workers and on an increase in the incidence of brain tumors in rats exposed to acrylonitrile by the inhalational and oral route.

The International Agency for Research on Cancer (3) classified acrylonitrile as a group 2A carcinogen (probable human carcinogen) based on sufficient evidence from laboratory animals and limited evidence from humans. The Health Council of The Netherlands (4) determined that acrylonitrile was carcinogenic in laboratory animals, but the evidence for humans was very weak.

The purpose of this paper is to present the overall toxicologic profile of acrylonitrile with the aim of elucidating the mechanism by which acrylonitrile is carcinogenic in rats.

Toxicokinetics

The data presented in this section have been taken from references 5—7 and from an unpublished report (Bos PMJ. The health-based recommended occupational exposure limit for acrylonitrile: draft report from the Dutch expert committee on occupational standards of the directorate-general of labor. The Hague, 1993:62 p).

Following inhalational or oral exposure, acrylonitrile absorption was shown to be rapid and extensive (90—98%), and distribution appeared rapidly throughout the body, with little significant accumulation in a particular organ. Seventy-two hours after the oral administration of radiolabeled acrylonitrile to F344 rats and B6C3F1 mice, the recovery of radioactivity ranged from 79% to 94% in urine and from 2% to 8% in feces.

The biotransformation of acrylonitrile occurs via 2 pathways: (i) conjugation with glutathione (GSH) and (ii) oxidation by cytochrome P450, resulting in the formation of the epoxide 2-cyanoethylene oxide (CEO). CEO is mutagenic and reacts much faster with DNA (deoxyribonucleic acid) than acrylonitrile. Therefore CEO is thought to play an important role in the carcinogenic properties of acrylonitrile.

In vitro experiments with liver microsomes have shown that the hepatic oxidation of acrylonitrile to CEO is high-

1 Toxicology Division of TNO Nutrition and Food Research Institute, Zeist, The Netherlands.

Reprint requests to: Dr Ruud A Woutersen, Toxicology Division, TNO Nutrition and Food Research Institute, PO Box 360, 3700 AJ Zeist, The Netherlands. [e-mail: Wouterson@voeding.tno.nl]

er in mice than in rats. The rate of CEO formation in human liver microsomes is similar to that in rat liver microsomes. In rodents, the detoxification of CEO occurs predominantly via GSH conjugation, whereas in humans detoxification via epoxidehydrolase plays an important role. Despite the higher rate of CEO formation in mouse liver microsomes, the concentration of CEO in blood is higher and CEO is detectable for a longer period in rats than in mice.

Excretion studies with rats have not always revealed uniform results. The excretion pattern is determined by the route of administration and by the dose. At higher acrylonitrile loads, the GSH pool can be depleted and lead to a relatively lower excretion of mercapturic acids and an increased excretion of glucuronides. Roughly 60% to almost 100% of administered acrylonitrile is excreted via the urine. Excretion in feces is of minor importance. Urinary analyses after the oral administration of acrylonitrile have revealed that the ratio of metabolites derived from CEO versus direct conjugation with GSH is about 2-fold higher in mice than in rats. These differences in metabolism may be responsible for the greater acute toxicity (see the section Acute Toxicity under Laboratory Animal Studies) of orally administered acrylonitrile in mice than in rats.

Mutagenicity and genotoxicity

Acrylonitrile has been found to be weakly mutagenic in the presence of metabolic activation in reverse mutation assays in several strains of *Salmonella typhimurium*. Acrylonitrile also appears to be weakly mutagenic in the TK6 human lymphoblast system, but only in the presence of metabolic activation and at a cytotoxic concentration (8). The epoxide metabolite of acrylonitrile, 2-cyanoethylene oxide (CEO), is a direct-acting mutagen.

A chromosome-damaging effect was determined in vitro in the micronucleus test on Chinese hamster ovary (CHO) cells and in chromosome aberration tests on CHO cells, as well as in liver and lung fibroblasts of the Chinese hamster. No chromosome damage was shown in a chromosome aberration test on rat hepatocytes and in the micronucleus test on human bronchial epithelium cells (9).

Most of the in vitro sister chromatid exchange (SCE) tests on rat hepatocytes and human lymphocytes have been negative (9). In vitro SCE tests on CHO cells are positive, especially after metabolic activation. The in vivo chromosome aberration tests on the mouse and rat, the micronucleus test on the mouse, and the dominant-lethal tests on the rat and mouse are all negative (9).

The dependence of the mutagenicity (and carcinogenicity) of acrylonitrile on the formation of the epoxide CEO has been a matter of debate in the scientific literature (8, 10, 11). The results of the mutagenicity and genotoxicity tests indicate that acrylonitrile itself hardly, or not at all, interacts with DNA and that the DNA-active compound is the epoxide CEO. This hypothesis is in accordance with the observations that acrylonitrile is genotoxic mainly after metabolic activation. The negative results obtained with in vivo genotoxicity tests might be explained by the effective detoxification of the epoxide CEO via GSH conjugation.

Hogy & Guengerich (10) found that after a single oral exposure of rats to a nearly lethal dose of 50 mg/kg, acrylonitrile caused an increase in unscheduled DNA synthesis in the liver. A single intraperitoneal CEO dose of 6 mg/kg was found to form guanine adducts [characterized as N7-(2-oxoethyl)-guanine] in rat liver DNA at very low levels (≈ 1—3 alkylations per 10^8 DNA bases). The significance of this adduct for the mutagenicity of CEO is questionable.

Table 1. Acute toxicity of acrylonitrile [data obtained from reference 5 unless otherwise stated]. (LD_{50} = median lethal dose)

Species	Route	Toxicity	
Mouse	Inhalation	LC_{50}	300 mg (m^3·4h)
Rat	Inhalation	LC_{50}	470 mg (m^3·4h)
Guinea pig	Inhalation	LC_{50}	990 mg (m^3·4h)
Mouse	Oral	LD_{50}	25—48 mg/kg
Guinea pig	Oral	LD_{50}	50—85 mg/kg
Rat	Oral	LD_{50}	72—186 mg/kg
Rabbit	Oral	LD_{50}	93 mg/kg
Mouse	Intraperitoneal	LD_{50}	47—50 mg/kg
Rat	Intraperitoneal	LD_{50}	65—100 mg/kg
Mouse	Subcutaneous	LD_{50}	25—50 mg/kg
Mouse	Subcutaneous	LD_{50}	35 mg/kg[a]
Hamster	Subcutaneous	LD_{50}	60 mg/kg[a]
Rat	Subcutaneous	LD_{50}	80—96 mg/kg
Rat	Subcutaneous	LD_{50}	100 mg/kg[a]
Guinea pig	Subcutaneous	LD_{50}	130 mg/kg
Rat	Percutaneous	LD_{50}	148—282 mg/kg
Rabbit	Percutaneous	LD_{50}	226 mg/kg
Guinea pig	Percutaneous	LD_{50}	200—690 mg/kg
Rabbit	Intravenous	LD_{50}	69 mg/kg
Guinea pig	Intravenous	LD_{50}	72 mg/kg

[a] Taken from reference 12.

Laboratory animal studies

Acute toxicity

The acute toxicity of acrylonitrile for different species is presented in table 1 (5, 12). The values for the oral median lethal dose (LD_{50}) for the various species range from 25 to 186 mg/kg of body weight. The sensitivity decreases in the order mouse, guinea pig, rabbit, rat. The LD_{50} values for intravenous, intraperitoneal, or subcutaneous application are similar to those for oral administration. The dermal LD_{50} values range from 148 to 693 mg/kg of body weight for rat, guinea pig, and rabbit. Rat is the most sensitive. The 50% lethal concentrations (LC_{50}) after 4 hours of

exposure lie in the concentration range of 300—990 mg/m^3. The sensitivity decreases in the order dog, mouse, rabbit, cat, rat, guinea pig.

Independent of the application mode, the biological effects after a lethal dose of acrylonitrile are excitability, convulsions, hind-leg incoordination, paralysis, apnea, respiratory disturbances until respiratory arrest, reddening of the skin, and lacrimation. Target organs in acute toxicity are the gastrointestinal tract (bleeding), the adrenals (hemorrhagic necrosis), the brain (edema), and the lungs (edema).

Irritation and sensitization

Reactions of shaven rabbit skin after exposure to acrylonitrile for 15 minutes or 20 hours comprise redness and swelling after 15 minutes and necrosis of tissue after exposure for 20 hours. Acrylonitrile causes opacity of the cornea and inflammation of the iris and the conjunctivae of rabbit eyes (13). According to the evaluation criteria of Draize, acrylonitrile is considered to be strongly irritating to the skin and the eye.

Short-term toxicity

Dogs appear to be the most sensitive species in short-term respiratory exposure to acrylonitrile (14). Exposure of dogs to 54 ppm (117 mg/m^3, 6 h/d, 5 d/week for 13 weeks) was lethal, whereas no effects on body or organ weight was observed at an exposure level of 24 ppm (52 mg/m^3). Mice and rats exposed to 24 or 54 ppm of acrylonitrile have not demonstrated an effect on body or organ weight (15). The acrylonitrile concentration of 24 ppm is considered the NOAEL (no-observable effect level) for dogs in short-term respiratory exposure. The difference between the lethal concentration (54 ppm) and the NOAEL (24 ppm), however, is remarkably small.

In the rat, a 90-day acrylonitrile uptake with drinking water causes body weight retardation accompanied by reduced feed and water uptake and increased relative liver weights in the 210 and 500 ppm dosage groups (17—42 mg/kg body weight). The NOAEL was found to be 85 ppm in drinking water (8 mg/kg body weight). In a 90-day study with B6C3F1 mice exposed to acrylonitrile by gavage, the NOAEL was established to be greater than 12 mg(kg · days). This NOAEL of 12 mg/(kg · days) is surprisingly high in comparison with the LD_{50} of 25 to 46 mg/(kg · days) observed for mice. The relative insensitivity of mice in this study might be related to the route of administration. In cases of exposure by gavage, the direct metabolism of acrylonitrile via GSH to cyanoethyl mercapturic acid (excreted in urine) might be the most important detoxification pathway, whereas, if exposure of the animals is via drinking water or inhalation, a greater part of the acrylonitrile will be metabolized via CEO.

In a 6-month drinking water study with dogs, 5 of 8 animals of the 18 mg/(kg · days) group died. Apart from mortality, increased relative kidney weights and reduced relative brain weights were observed; a concentration of 8 mg/(kg · days) was tolerated without symptoms.

Long-term toxicity and carcinogenicity

Several chronic toxicity and carcinogenicity bioassays have been conducted on Sprague Dawley rats or Fischer 344 rats through multiple routes (inhalation, gavage administration, drinking water), but not on any other species. The results of these studies have been reviewed and summarized previously by the Advisory Committee on Existing Chemicals of Environmental Relevance of the German Chemical Society (14) and more recently by the Toxicology Excellence for Risk Assessment for the Acrylonitrile Group (16).

Treatment-related neoplasms have been reported in all the carcinogenicity studies performed. A high incidence of astrocytomas in the brain and spinal cord was the most consistent finding in these studies. Statistically significant increases in brain tumor incidences were found in Fischer 344 rats exposed to 10 ppm of acrylonitrile via drinking water for 24 months (17). In another 2-year drinking water study with Sprague Dawley rats (Spartan substrain), statistically significant increased incidences of astrocytomas were found at levels of 35 ppm and above (18).

The lowest dose associated with a statistically significantly increased incidence of astrocytomas after inhalation exposure of Sprague Dawley rats (6 h/d, 5 d/week for 104 weeks) was 20 ppm for females and 80 ppm for males (19).

Zymbal gland tumors were the second most common tumor type reported after long-term acrylonitrile administration to rats. Furthermore, tumors of the small intestine (cystadenocarcinomas), mammary gland (adenocarcinomas), tongue and nonglandular stomach (papillomas and squamous cell carcinomas) were reported to be increased after exposure of rats to acrylonitrile (18, 17, 20—24). Nonglandular stomach tumors were observed in rats after oral exposure to acrylonitrile only, whereas all of the other aforementioned tumors developed in rats after both oral and inhalational exposure to acrylonitrile.

In some studies, the incidences of pituitary adenomas, adrenal pheochromocytomas, and tumors of the thyroid and pancreas were decreased in animals exposed to high levels of acrylonitrile in comparison with the incidences of controls. This result has been considered to be attributed to the treatment-related early mortality observed in these groups.

Reproduction toxicology

Murray et al (25) administered acrylonitrile by inhalation (0, 87, or 174 mg/m^3 for 6 h/d) or by gavage (0, 10, 25, or 65 mg/kg body weight · day) to pregnant Sprague-Dawley rats on days 6 to 15 of gestation. The 65 mg/kg exposure level was toxic to the mother animals and resulted in

embryotoxicity and malformations in the fetuses (short-tail, short trunk, missing vertebrae, and an aortic arch turning to the right).

There is no evidence of teratogenic effects below maternal toxic levels.

Tandon et al (26) noticed a decreased sperm count in CD-I mice daily administered acrylonitrile (10 mg/kg body weight · d) by gavage for 60 days.

Abdel Naim et al (27) exposed rats by gavage to acrylonitrile (0, 10.5, 23 and 46 mg/(kg body weight · d) for 4 weeks. Acrylonitrile induced a dose-dependent decrease in body and testis weight. Sperm count and sperm motility were significantly decreased. Microscopic examination of the testes revealed a decreased number of spermatocytes and spermatids at 23 and 46 mg/(kg body weight · d), which is close to the LD_{50} for rats. Such effects were not found in long-term inhalation and drinking water studies, where exposure to acrylonitrile was spread over a much longer period of the day than when given instantly by gavage.

Mode of action

Acrylonitrile has been shown to be weakly genotoxic, primarily through its metabolism to CEO. (See the section on mutagenicity and genotoxicity.) Acrylonitrile itself hardly, if at all, interacts with DNA. The epoxide CEO is a direct-acting mutagen which binds DNA with a much greater affinity than acrylonitrile. Adducts on guanine have been detected at very low levels in the liver of rats treated with CEO, but the significance of these adducts to the carcinogenic process is not clear. It can be concluded from the data presented in this paper that acrylonitrile is weakly genotoxic but the exact mechanism of the carcinogenicity is unknown. Acrylonitrile at a nearly lethal dose has been found to interact with DNA in the liver and stomach, but an interaction of acrylonitrile with brain DNA after short- and long-term exposure has not been demonstrated. Formation of 8-oxodeoxyguanosine in brain DNA, reflecting oxidative tissue damage, was observed in rats acutely or chronically exposed to acrylonitrile (28). This finding may point to an epigenetic rather than a genetic mechanism involved in the induction of astrocytomas in the brain of rats exposed to acrylonitrile.

Health risk assessment with respect to carcinogenicity

The Toxicology Excellence for Risk Assessment Group (16) has calculated quantitative risk estimates for inhalation exposure based on the long-term rat bioassay reported by Quast et al (19). They modeled the tumor incidence data for astrocytomas (benign and malignant combined) in the observable range using a polynomial model (ie, the linearized multistage model). From this model, the ED_{10} concentration associated with a 10% increase in tumor incidence and the LED_{10} (the 95% lower confidence limit of the ED_{10}) were determined. Linear extrapolation from these 2 points to the origin were done to estimate risk levels at lower concentrations. The authors concluded that the data on the mechanism of action are currently insufficient to rule out the possibility that a linear dose-response exists for acrylonitrile. Based on the animal model, the lifetime risk from continuous exposure to an acrylonitrile concentration of 1 mg/m^3 was determined to be in the range of $1.1 \cdot 10^{-5}$ (based on the LED_{10}) to $8.2 \cdot 10^{-6}$ (based on the ED_{10}).

According to these data, the calculated health-based occupational cancer risk value, which is associated with excess cancer mortality levels of 4 per 1000 as a result of worklife exposure (during 40 years, 48 weeks/year, 5 d/week, 8 h/d) can be calculated to be 4.1 mg/m^3 for acrylonitrile.

Recommendations for further research

From the data presented in this paper, it can be concluded that acrylonitrile is carcinogenic in rats when the exposure is oral or inhalational. However, information on other mammalian species is lacking, and, moreover, the exact mechanism of the carcinogenic process is unclear. In 1996 a study on the carcinogenicity of acrylonitrile given to mice by gavage was started by the National Toxicology Program in the United States.

In this respect, the implications of the interspecies variation and the route of administration in the metabolism of acrylonitrile needs further investigation. The long-term carcinogenicity study with mice, as well as studies aimed at elucidating the mechanism of brain tumor induction (genetic versus epigenetic), may contribute to the understanding of the mechanism by which acrylonitrile induces brain tumors in rats.

Furthermore, it may be worthwhile to perform inhalation cancer risk assessment using the incidence of astrocytomas only (thus excluding putative preneoplastic glial cell proliferation), adjusted for intercurrent mortality.

References

1. US Environmental Protection Agency (EPA). The risk assessment guidelines of 1986. Washington (DC): Office of Health and Environmental Assessment, 1986. EPA/600/8—87/045.

2. US Environmental Protection Agency (EPA). Integrated risk information system [online]. Washington (DC): Office of Research and Development, 1996.
3. International Agency for Research on Cancer (IARC). Acrylonitrile, acrylic and modacrylic fibers, and acrylonitrile-butadiene-styrene and styrene-acrylonitrile copolymers. Lyon: IARC, 1979:73—113. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, no 19.
4. Gezondheidsraad. Advies inzake acrylonitril in de buitenlucht op basis van een criteriadocument over deze stof. 's Gravenhage: Gezondheidsraad, 1985:53 p.
5. World Health Organization (WHO). Acrylonitrile. Geneva: WHO, 1983:125 pp. Environmental health criteria, no 28.
6. Linhart I, Smejkal J, Novák J. N-acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine, a new urinary metabolite of acrylonitrile and oxiranecarbonitrile. Arch Toxicol 1988;61(6):484—8.
7. Fennell TR, Kedderis GL, Sumner SCJ. Urinary metabolites of [1,2,3-^{13}C]acrylonitrile in rats and mice detected by ^{13}C nuclear magnetic resonance spectroscopy. Chem Res Toxicol 1991;4:678—87.
8. Recio L, Skopek TR. Mutagenicity of acrylonitrile and its metabolite 2-cyanoethylene oxide in human lymphoblasts in vitro. Mutat Res 1988;206(2):297—305.
9. Ashby J, de Serres FJ, Draper M, Ishidate M Jr, Margolin BH, Matter BE, et al, editors. Evaluation of short-term tests for carcinogens: report of the International Programme on Chemical Safety's collaborative study on in vitro assays. Prog Mutat Res 1985:5.
10. Hogy LL, Guengerich FP. In vivo interaction of acrylonitrile and 2-cyanoethylene oxide with DNA in rats. Cancer Res 1986;46:3932—8.
11. Kedderis GL, Batra R. Species differences in the hydrolysis of 2-cyanoethylene oxide, the epoxide metabolite of acrylonitrile. Carcinogenesis 1993;14:685—9.
12. Cote IL, Bowers A, Jaeger RJ. Effects of acrylonitrile on tissue glutathione concentrations in rat, mouse, and hamster. Res Commun Chem Pathol Pharmacol 1984;43:507—10.
13. Brittelli HR, Morrow RW. Eye irritation test in rabbits. DuPont Haskell Laboratory, 1975. DuPont Haskell laboratory report, no 714—75.
14. GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance, editor. Acrylonitrile: BUA report 142 (August 1993). Stuttgart: S Hirzel, Wissenschaftliche Verlagsgesellschaft, 1995.
15. Brewer WE. 90-day subacute vapor inhalation toxicity study with acrylonitrile in beagle dogs, albino rats, and albino mice. 1976. Industrial biotest report, no 74—42, prepared for the Monsanto company. Cited in reference 5.
16. Toxicology Excellence for Risk Assessment (TERA). Acrylonitrile: inhalation cancer risk assessment. Cincinnati (OH): TERA, 1997:63 pp. Prepared for The Acrylonitrile Group by Toxicology Excellence for Risk Assessment.
17. Bio Dynamics Inc. A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered to Spartan rats in the drinking water. St Louis (MO): Monsanto Company, 1980. Final report, vol I.
18. Quast JF, Wade CE, Humiston CG, Carreon RM, Hermann EA, Park CN, et al. A two year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats. Midland (MI): Dow Toxicology Research Laboratory, 1980.
19. Quast JF, Schuetz DJ, Balmer MF, Gushow TS, Park CN, McKenna MJ. A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Midland (MI): Dow Toxicology Research Laboratory, 1980.
20. Bio Dynamics Inc. A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered in the drinking water to Fischer 344 rats. St Louis (MO): Monsanto Company, 1980. Final report, vol I.
21. Bio Dynamics Inc. A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered by intubation to Spartan rats. St Louis (MO): Monsanto Company, 1980. Final report, vol I.
22. Bigner DD, Bigner SH, Burger PC, Shelburne JD, Friedman HS. Primary brain tumors in Fischer 344 rats chronically exposed to acrylonitrile in their drinking-water. Food Chem Toxicol 1986;24:129—37.
23. Gallagher GT, Maull EA, Kovacs K, Szabo S. Neoplasms in rats ingesting acrylonitrile for two years. J Am Med Coll Toxicol 1988;7:603—15.
24. Maltoni C, Ciliberti A, Cotti G, Perino G. Long-term carcinogenicity bioassays on acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. Ann NY Acad Sci 1988;534:179—202.
25. Murray FJ, Schwetz BA, Nitschke KD, John JA, Norris JM, Gehring PJ. Teratogenicity of acrylonitrile given to rats by gavage or inhalation. Food Cosmet Toxicol 1978;16:547—51.
26. Tandon R, Saxena DK, Chandra SV, Seth PK, Srivastava SP. Testicular effects of acrylonitrile in mice. Toxicol Lett 1988;42(1):55—63.
27. Abdel Naim AB, Hamada F, Abdel Aziz AH, Ahmed AE. Acrylonitrile (VCN)-induced testicular toxicity in the rat. Toxicologist 1994;14(1):87.
28. Whysner J, Conaway CC, Verna LK, Rosen JE, Prahalad AK, Richie Jr JP, et al. Formation of 8-oxodeoxyguanosine in brain DNA of rats exposed to acrylonitrile. Toxicologist 1996;30:67.