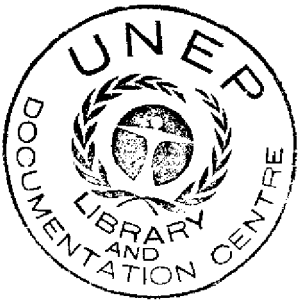
Environmental Health Criteria 28

ACRYLONITRILE

Published under the joint sponsorship of
the United Nations Environment Programme,
the International Labour Organisation,
and the World Health Organization



World Health Organization
Geneva, 1983

The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

ISBN 92 4 154088 5

PRINTED IN FINLAND

83/5963 VAMMALA 5500

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluation of the conclusions contained in the criteria documents.

TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR ACRYLONITRILE

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Further to the recommendations of the Stockholm United Nations Conference on the Human Environment in 1972, and in response to a number of World Health Assembly resolutions (WHA23.60, WHA24.47, WHA25.58, WHA26.68) and the recommendations of the Governing Council of the United Nations Environment Programme, (UNEP/GC/10, July 3 1973), a programme on the integrated assessment of the health effects of environmental pollution was initiated in 1973. The programme, known as the WHO Environmental Health Criteria Programme, has been implemented with the support of the Environment Fund of the United Nations Environment Programme. In 1980, the Environmental Health Criteria Programme was incorporated into the International Programme on Chemical Safety (IPCS). The result of the Environmental Health Criteria Programme is a series of criteria documents.

The Institute of Hygiene and Epidemiology (Director, Professor Bohumír Rosický), Prague, was responsible, as a Lead Institution of the IPCS, for the preparation of the first and second drafts, which were written and coordinated by Dr I. Gut and Dr J. Kopecký of that Institute.

The Task Group for the Environmental Health Criteria for Acrylonitrile met in Prague in the Institute of Hygiene and Epidemiology from 4-8 July 1983. The meeting was opened by Professor B. Rosický, and Dr M.H. Draper welcomed the participants and representatives of the organizations on behalf of the three organizations co-sponsoring the IPCS (UNEP/ILO/WHO). The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks of exposure to acrylonitrile.

The efforts of all who helped in the preparation and the finalization of the document are gracefully acknowledged.

* * *

Partial financial support for the publication of this criteria document was kindly provided by the United States Department of Health and Human Services through a contract from the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA - a WHO Collaborating Centre for Environmental Health Effects.

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH

1.1 Summary

1.1.1 Properties and analytical methods

Acrylonitrile (CH_2=CH-C≡N) is a volatile, colourless, flammable liquid with a sweet characteristic odour. It is used in the production of acrylic and modacrylic fibres, resins and rubbers, and as a chemical intermediate. It has been employed as a fumigant. Exposure to both the vapour and the liquid can occur at the workplace, the highest atmospheric concentrations occurring in acrylic fibre production.

For the control of exposure to acrylonitrile at the workplace, sampling should preferably be from the breathing zone of the worker; active and passive sampling techniques are available.

The most widely used analytical techniques are the gas chromatographic techniques; these are particularly sensitive if nitrogen-sensitive and specific sensors are used. High-pressure liquid chromatographic, infra-red, and colorimetric methods may be useful, where gas chromatography is not available. Methods have been developed for the determination of acrylonitrile in blood, food, water, etc. Determination of acrylonitrile-derived mercapturic acids in urine may prove to be of value for the biological monitoring of exposure.

1.1.2 Sources of exposure

Acrylonitrile is emitted from industrial plants in the form of vapours and in aqueous effluents; exposure of the population living near plants cannot therefore be excluded. The total emissions from acrylonitrile plants have been estimated to be about 2.2% of total production, but these figures have decreased recently. Polymers contain various concentrations of free acrylonitrile; when used for packaging in the food industry, minute amounts of the monomer may pass into the food. Acrylonitrile may also enter the environment accidentally, during its storage and transport.

1.1.3 Industrial and environmental levels of exposure

Contamination of water and food is possible but, with the exception of the contamination of water supplies through accidental spillage, levels of exposure would be low. The highest potential for exposure is at the workplace, both through inhalation of vapour and contamination of the skin by liquid acrylonitrile.

1.1.4 Monitoring of acrylonitrile uptake

The most significant uptake of acrylonitrile vapour is through the respiratory tract. Exposure is commonly monitored by determining the time-weighted average atmospheric concentrations.

Estimation of acrylonitrile-derived mercapturic acids in urine is a promising method for the biological monitoring of exposure, but further validating studies are needed.

1.1.5 Absorption, distribution, biotransformation, and elimination

In animals, acrylonitrile is readily absorbed both through the skin and by inhalation. Systemic and even fatal effects are possible via these routes.

The distribution of acrylonitrile within the animal body is fairly uniform. There are no indications of accumulation in animal tissues following prolonged exposure.

At least 10 different metabolites of acrylonitrile have been identified. Mercapturic acids are the major metabolites of acrylonitrile *in vivo*. Urinary excretion of acrylonitrile-derived mercapturic acids is proportional to the internal concentration of acrylonitrile.

Elimination of acrylonitrile, as such, in expired air is negligible, but a small percentage is eliminated in the urine.

1.1.6 Effects on experimental animals

Acrylonitrile induces a variety of toxic effects. Effects due to over-exposure are non-specific and mainly related to the gastro-intestinal and respiratory tracts, the central nervous system, and the kidneys. Respiratory distress, lethargy, convulsions, and coma occur with lethal or near-lethal exposures (7500 mg/m^3, inhalation). Dogs are most sensitive, and rats least sensitive to acrylonitrile, with mice, guinea-pigs, cats, and monkeys in an intermediate position. However, the information available from these studies is too fragmentary to indicate clear no-observed-adverse-effect levels.

Extensive dermal exposure to the liquid may be lethal. At lower exposures, irritation of the skin and mucous membranes can occur.

The most typical biochemical changes caused by acrylonitrile are inhibition of sulfhydryl-dependent enzymes (lactate dehydrogenase, LDH (EC 1.1.1.27), sorbitol dehydrogenase, SDH (EC 1.1.1.14), pyruvate oxidase (EC 1.2.3.3)) and a reduction in the concentrations of glutathione and protein sulfhydryls in the blood and various

organs, resulting in a disturbance of glucose utilization. The cyanide generated causes inhibition of cytochrome oxidase (EC 1.9.3.1) but this seems to be of less significance than the above-mentioned metabolic disturbances, at low exposure levels.

Exposure to some organic solvents in addition to acrylonitrile may significantly enhance its toxic effects.

Acrylonitrile can cause embryotoxic and teratogenic effects, but only at levels near the toxic dose level for the specific experimental animal.

It is probable that acrylonitrile is not mutagenic itself, but that its metabolites are responsible for the positive effects in various test systems. It is mutagenic in in vitro systems (bacterial tests and cell cultures), but not in in vivo systems, such as the dominant lethal assay.

On the basis of the results of several animal studies, using a wide dose-range, there is sufficient evidence to suggest that acrylonitrile is a carcinogen in the rat.

1.1.7 Effects on man

Symptoms of over-exposure in man are non-specific. They are related to the gastrointestinal and respiratory tracts, and to the central nervous system and include headache, insomnia, nausea, vomiting, diarrhoea, fatigue, mild jaundice, and irritation and inflammation of the respiratory tract and mucous membranes. In more severe cases, unconsciousness and convulsions may occur. Fatalities have been reported following exposure to acrylonitrile, especially following its use as a fumigant. Dermal exposure, especially to liquid acrylonitrile, may cause irritation, erythema, and blisters. Toxic and allergic dermatitis can occur.

While a correlation between exposure to acrylonitrile and the incidence of cancer in man has not been demonstrated conclusively in human epidemiological studies, the findings are not incompatible with this supposition. Thus, there is no reason to disregard the evidence that has been provided by animal studies.

It follows that exposure to acrylonitrile should be kept as low as possible at the workplace and in the general environment, and that skin contact with liquid acrylonitrile should be avoided.

1.2 Recommendations for Further Research

The Task Group noted that valuable information from industry, while available to national and international bodies, had not been published. This greatly reduces the

value of these studies, as they are unavailable for peer review and critical examination by the scientific community.

The Group recommended the following studies:

(a) Improvement and validation of passive sampling techniques with special attention to interfering substances;

(b) Validation of the measurement of acrylonitrile and acrylonitrile-derived mercapturic acids in urine as methods for biological monitoring for workplace exposure, with regard to analytical aspects and sampling conditions;

(c) Investigation of the environmental fate of acrylonitrile including photochemical degradation;

(d) Further investigation of the mechanisms of action and the nature of acute and chronic toxic effects in conditions relevant to human exposure;

(e) Studies on the carcinogenicity of acrylonitrile in relation to animal species other than the rat;

(f) Further investigation of the metabolism and toxicokinetics of acrylonitrile in different animal species, in order to obtain information that will assist in the interpretation of biological monitoring data in man;

(g) Further examination of the immunological aspects of the action of acrylonitrile in man and animals;

(h) Further studies on the effects of acrylonitrile on reproduction;

(i) Investigations on reproductive outcome and mutagenicity in human beings occupationally exposed to acrylonitrile.

Epidemiological data with good indications of past and present exposure levels should be available, to ensure an adequate health risk evaluation.

2. PROPERTIES AND ANALYTICAL METHODS

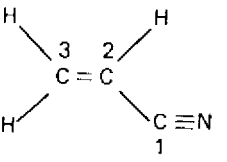
2.1 Physical and Chemical Properties of Acrylonitrile

2.1.1 Physical properties

Acrylonitrile (CH_2=CH-C≡N) is a volatile, colourless, flammable liquid with a sweet, characteristic odour. It is slightly soluble in water and miscible with most organic solvents (American Cyanamid, 1959). The vapours are explosive, cyanide gas being produced. The explosive range in air at 25 °C has a lower limit of 3.05%, and an upper limit of 17.0%, by volume (Patty, 1963). The olfactory threshold level for acrylonitrile averages 40.4 mg/m^3 (18.6 ppm) and ranges from 0.007 to 109.4 mg/m^3 (0.0031 to 50.4 ppm) (Baker, 1963). Important physical constants and properties of acrylonitrile are summarized in Table 1.

2.1.2 Chemical properties

Structural formula:



WHO 83933

Synonyms: cyanoethylene, 2-propenenitrile, vinyl cyanide.

CAS Registry Number: 107-13-11.

The reactions of acrylonitrile involve the double bond (C=C) and/or the cyano group (CN) (American Cyanamid, 1959). It polymerizes to polyacrylonitrile, and copolymerizes with, e.g., styrene, butadiene, esters of acrylic or methacrylic acid, to form various resins, nitrile rubber, and acrylic and modacrylic fibres. Hydration produces acrylamide or acrylic acid and esterification the corresponding acrylic esters. Reductive coupling produces adiponitrile. With compounds containing active hydrogen(s) (AH molecules such as the biologically-important compounds containing the nucleophilic -CH, -NH, and -SH groups), cyanoethylation takes place:

$$A\text{-}H + CH_2 = CH\text{-}CN + A\text{-}CH_2CH_2CN$$

Table 1. Physical properties of acrylonitrile[a]

appearance	colourless liquid
boiling point	77.3 °C at 760 mm pressure
density	0.8060 (20 °C), 0.8004 (25 °C)
flash point (Tag open cup) (closed cup)	0 °C -4.4 °C
freezing point	-82.55, ± 0.05 °C
ignition temperature	481 °C
relative molecular mass	53.06
octanol/H_2O partition coefficient	0.12[b]
odour	faintly pungent
refractive index	n_D^{25} = 1.3893
% solubility in water[c]	7.28 (0 °C) 7.35 (10 °C) 7.96 (30 °C)
vapour pressure (mm Hg)	50 (18.7 °C) 100 (23.6 °C) 250 (45.5 °C) 500 (64.7 °C) 760 (77.3 °C)
partial vapour pressure water azeotrope	$\log P = 7.91m - \frac{1664.7}{T}$ (i.e., 88 mm at 20 °C)
Conversion factor for vapour (25 °C; 760 mm Hg)	1 mg/m^3 = 0.4605 ppm 1 ppm = 2.171 mg/m^3 1 mg/litre water = 1 ppm

[a] From: American Cyanamid (1959, 1974).
[b] From: Dorigan et al. (1976); ancillary of -0.92.
[c] Acrylonitrile is miscible with most organic solvents.

(American Cyanamid, 1959). This reaction is of particular importance in relation to its fate in biological systems; covalent binding of acrylonitrile to the tissue components has been demonstrated (section 7.1.3.3). Direct oxidation of acrylonitrile with hydroperoxide compounds affects the cyano group of acrylonitrile, although in biological systems, it is probable that oxidation of the double bond to the oxirane, glycidonitrile (CH_2—CH–CN) occurs (Kopecký et al., 1980a,b).

There have not been any experimental studies but, as a reactive olefine, it would be expected that acrylonitrile would be oxidized in the atmosphere under the influence of ultraviolet radiation (UVR) or by reactive oxygen species (atomic oxygen, OH radicals, ozone). The atmospheric half-life of acrylonitrile is estimated to be 9-10 h (Suta, 1979).

Technical-grade acrylonitrile is more than 99% pure. Except for water, impurities and stabilizers are present at mg/kg levels only. Possible contaminants are shown in Table 2. Spontaneous explosive polymerization of pure acrylonitrile may occur, in the absence of oxygen, on exposure to visible light or alkali (DuPont, 1977). A yellow colour may slowly develop on standing, particularly after excessive exposure to light. Water improves the stability of acrylonitrile, and the technical-grade product is stabilized against self-polymerization and colour formation by the addition of hydroquinone monomethyl ether and water.

2.2 Analytical Methods

In this section, sampling methods, sample storage, and analytical methods for determining acrylonitrile and its metabolites are discussed. The only breakdown products considered are those detected *in vivo*, as these are the only ones of importance for assessing levels of exposure to acrylonitrile.

2.2.1 Sampling methods

Sorption tubes are widely used for sampling acrylonitrile in air, because samples can be taken over a prolonged period from the breathing zone of the worker. The solid sorbent gas samplers have been critically reviewed by Crisp (1980). Of the solid sorbents, activated charcoal, porous polymers, or silica gel are most commonly used. Adsorbed acrylonitrile is later desorbed, generally by a solvent (methanol or carbon disulfide) or thermally, and determined by gas chromatography. Several devices have been developed for sampling workplace air. A sorbent sampling tube fastened to the worker's shoulder and a pump fastened to the belt may be worn for a whole working shift without discomfort. Muhtarova (1977) described significant differences between the results of static sampling and personal monitoring in determining acrylonitrile exposure in workers. Personal monitoring gives a better indication. Area concentrations can be determined by detector tubes, to give an immediate indication of the level (CIA, 1978; Grote et al., 1978).

Table 2. Specifications for acrylonitrile from two producers[a]

Specifications	DuPont	Monsanto
acetone, mg/kg max.	n.r.[b]	300
acetonitrile, mg/kg max.	500	500
aldehydes, as acetaldehyde mg/kg max.	50	50
iron, mg/kg max.	0.1	0.2
hydrocyanic acid, mg/kg max.	10	5
peroxides, as hydrogen peroxide, mg/kg max.	0.3	1.0
water, %	2.5-4.5	2.5-4.5
inhibitor, MEHQ[c], mg/kg	35 - 50	35 - 50
acidity, as acetic acid, mg/kg max.	35	20
pH, 5% aqueous solution	5.5-7.5	n.r.[b]
non-volatile matter, mg/kg max.	100	100
refractive index at 25 °C	1.3880 - 1.3892	1.3880 - 1.3892
appearance	clear & free flowing	clear & free flowing

[a] From: DuPont (1977) and Monsanto (1977a).
[b] n.r. = not reported.
[c] MEHQ = hydroquinone monomethyl ether (methylhydroquinone).

In the widely-used NIOSH method S156 (NIOSH, 1976), a known volume of air is drawn through a charcoal tube (divided into 2 sections in order to check that the adsorption capacity has not been swamped), and the charcoal is desorbed by methanol for 30 min. This method was validated by NIOSH over a concentration range of 17.5-70.0 mg/m^3 (8.1-32.3 ppm) at 22 °C and 760 mm Hg using a 20-litre sample; the coefficient of variation was 0.073. However, the suspicion that acrylonitrile may be a human carcinogen (NIOSH, 1978) led to the need to determine lower concentrations of acrylonitrile in air. With a simple modification in method S156, using a desorbing solvent of 2% v/v acetone solution in carbon

disulfide, Gagnon & Posner (1979) were able to achieve a sensitivity of 1.1 mg/m^3 (0.5 ppm) based on an air sample volume of 15 litres. The samples are stable for at least a week, even in the absence of a stabilizer. A similar method, developed by the Midwest Research Institute for sampling air near acrylonitrile plants (Going et al., 1979), involves the use of charcoal tubes, sampling air at 1 litre/min, desorbing the sample with carbon disulfide, and analysing by gas chromatography. However, high humidity and interference from other substances can reduce collection efficiency on charcoal; these problems can be overcome by the use of porous polymer absorbents and thermal desorption techniques (Campbell & Moore, 1979; United Kingdom Health and Safety Executive, 1981).

While many industrial hygiene personal monitoring measurements have been carried out using these methods, over the last 3-4 years an increasing number of "passive" samplers (gas badges) (Silverstein, 1977) have been developed. The advantages of these devices are that there are no moving parts to break down, regular flow calibration is unnecessary, and no bulky, expensive pumps are required.

Benson & Boyce (1981) and Benson et al. (1981) described a passive dosimeter in which acrylonitrile was adsorbed on a porous polymer (Porapak ^{R}N) contained in a removable element, and determined by thermal desorption gas chromatography. It can be used satisfactorily for determining acrylonitrile concentrations in air under a range of atmospheric conditions, when working to a control limit of 8.7 mg/m^3 (4 ppm) but, at a concentration of 4.4 mg/m^3 (2 ppm), a 40% error has been reported. These devices are now considered to be as reliable as the more conventional pump and tube methods (Rose & Perkins, 1982).

The head-space sampling method is useful for the determination of residual acrylonitrile monomer in copolymers and by-products, since it is more sensitive (detection limit 1.1 mg/m^3 (0.5 ppm)) than direct injection (detection limit 21.7 mg/m^3 (10 ppm)) (Steichen, 1976). It involves the equilibration of a solid polymer with air in a closed vessel. Free monomer is partitioned between the polymer phase and the "head-space" air, and the monomer concentration in the head-space is then determined (Steichen, 1976). Oomens (1980) gives a detection limit for acrylonitrile of 0.02 mg/m^3 (0.01 ppm) with the aid of a similar method, applying the more sensitive and specific PND detector. The procedure has been used for determining the acrylonitrile monomer in copolymer solutions (McNeal & Breder, 1981), plastic packaging, and beverages (Gawell, 1979). Gawell's method is suitable for determining acrylonitrile at concentrations as low as 0.1 mg/kg, in plastics, and 0.005 mg/kg, in beverages. The method has also been used for determining acrylonitrile in food-

simulating solvents (US FDA, 1977a) and, with a detection limit of 0.5 mg/kg, in acrylonitrile-derived copolymers (Steichen, 1976).

Continuously recording gas chromatographic methods have been developed for monitoring atmospheric concentrations of acrylonitrile.

Samples of water containing acrylonitrile can be acidified by sulfuric acid to a pH ≤ 4 and then kept at 0 °C until analysed (Going et al., 1979).

2.2.2 Analytical methods for determinating acrylonitrile

Acrylonitrile can be determined using instrumental methods: gas chromatography, possibly high-pressure liquid chromatography, infrared spectroscopy, polarography, and chemical titrimetric and colorimetric methods.

(a) Gas chromatography

This is the most frequently used method for acrylonitrile determination, particularly in conjunction with the charcoal sampling method. A number of gas chromatographic procedures have been developed for different types of samples. Until recently, almost all involved flame ionization detection, but attention is now being paid to thermoionic nitrogen-selective detectors (Shevchik, 1976) in the determination of acrylonitrile (e.g., US FDA, 1977a; Gawell, 1979; McNeal & Breder, 1981).

Various column packings have been evaluated for the determination of acrylonitrile by gas chromatography, e.g., in the air (Parsons & Mitzner, 1975; Russell, 1975) (Table 3). Porous polymer column packings have the advantage of resolving acrylonitrile from methanol (frequently used to desorb acrylonitrile from charcoal) and of being useful for direct injection of aqueous acrylonitrile samples.

Examples of gas chromatographic methods for determining acrylonitrile in a variety of products and samples containing acrylonitrile are given in Table 4, together with the detection limits.

Borg-Warner Chemicals (1977) developed a continuous-recording gas chromatograph that reportedly detects acrylonitrile below 1.1 mg/m^3 (0.5 ppm). A portable gas chromatograph for the determination of acrylonitrile in air was developed by Vistron (personal communication, 1978) and a direct injection gas chromatograph for acrylonitrile determinations was tested by Union Carbide Corporation (1977); preliminary results indicate a detection limit below 2.2 mg/m^3 (1 ppm).

Table 3. Gas chromatographic conditions for acrylonitrile determination

Packing	Conditions	Comments	Reference
Tenax	80 °C, 15 cc/min N_2, -, 60 x 0.3 cm, Teflon	Used by American Cyanamid for water analysis	
0.4% Carbowax 1500 on Carbopax A	100 °C, 30 cc/min He, -, 80 x 0.3 cm, stainless steel	Head space analysis of residual monomer	Steichen (1976)
Porapak Q, 50/80 mesh	155 °C, 50 cc/min N_2, -, 120 x 0.6 cm stainless steel	NIOSH method for acrylonitrile in air	NIOSH (1976)
Porapak Q, 50/80 mesh	160 °C, 30 cc/min N_2, 3.2 min, 150 x 0.3 cm stainless steel	Poor resolution from methanol	Barrett (1974)
Porapak N, 50/80 mesh	170 °C, 40 cc/min N_2, 10.5 min, 270 x 0.3 cm stainless steel	Resolved from methanol	Barrett (1974)
Chromosorb 101, 50/60 or porous styrene divinyl benzene polymer	110 °C to 200 °C at 10 °C/min, 25 ml/min He, 240 x 0.3 cm stainless steel	ASTM approved method for nitriles in water	ASTM (1981)
Porapak Q, 50/80 mesh	156 °C, 50 cc/min He, 11.8 min, 360 x 0.3 cm stainless steel	Used with a trapping column for combustion effluents	Bellar & Sigsby (1980)
10% SP - 1000, 60/80 mesh supelcopore	150 °C, 45 cm/min	Acrylonitrile plus various organic vapours	Marano et al. (1978)

[a] Column temperature, carrier gas and flow rate, retention time, column parameters.

Table 4. Determination of acrylonitrile in different acrylonitrile-containing samples and products

Sample source	Detection limit	Reference
water solution	10 mg/kg	Ramstad & Nicholson (1982)
polyacrylonitrile	10 - 100 mg/kg	Reichle & Tengler (1968)
vinylidene chloride-acrylonitrile coated film	10 mg/kg	UK Ministry of Agriculture, Fisheries & Food (1982)
food samples	0.01 - 0.02 mg/kg	UK Ministry of Agriculture, Fisheries & Food (1982)
acrylic co-polymers	0.5 mg/kg 70 mg/kg	Steichen (1976) McNeal & Breder (1981)
carbonated beverage (simulated)	1 mg/kg	McNeal & Breder (1982)
fumigant residue in cereals & other foods	0.1 mg/kg	Heuser & Scudmore (1969)
air of acrylonitrile plants	n.s.	Cincolella et al. (1981)
acetone extract of styrene-acrylonitrile resins	1 mg/kg	US Consumer Product Safety Commission (1978)

n.s. = not stated.

(b) High-pressure liquid chromatography

A high-pressure liquid chromatograph method has been developed for the determination of residual acrylonitrile monomer in acrylic polymer and fibre (US Consumer Product Safety Commission, 1978). The acrylic polymer or fibre is heated above its glass transition temperature and refluxed continuously under water. The extract is distilled and analysed. No interference from contaminants has been noted.

(c) Infrared spectroscopy

Direct determination of acrylonitrile in air by IR spectroscopy, using wavelength 10.49 μm, 20 °C and 760 mm Hg, and a 250 cm gas cell, has been reported to have a detection limit of about 0.5 ppm (v/v). The equipment is expensive, requires skill to use, and is sensitive to physical damage. A portable IR analyser for "on-the-spot" detection of acrylonitrile in air, with a detection limit of 0.4 mg/m^3 (0.2 ppm), has been recommended by Jacobs & Syrjala (1978).

(d) Polarography

A polarographic method for the determination of acrylonitrile was first reported by Bird & Hale (1952). Berck (1960) used the method of Daues & Hamner (1957) to determine acrylonitrile residues in walnuts. Aqueous extracts of styrene-acrylonitrile copolymer (Petrova et al., 1972), the volatile fractions of styrene copolymer (Uhde & Koehler, 1967), and industrial waste water (Ponomarev et al., 1974) have also been analysed using polarography. A method developed by Rogaczewska (1964) had a sensitivity of 10 mg/litre and 40 mg/m^3 for the determination of acrylonitrile in solution and in air, respectively.

(e) Colorimetric methods

In one method, the acrylonitrile-containing sample is hydrolysed by a strong base to ammonia, which is determined by the Nessler reagent (Rogaczewska, 1965; Aarato & Bittera, 1972). The detection limit of this method is about 6 mg/m^3 (3 ppm) in air. A modification using hypochlorite and sodium salicylate has a detection limit of 0.5 mg/m^3 (Rogaczewska, 1976).

A modified hydrolytic method using hydrogen peroxide under acidic conditions has been developed for the determination of acrylonitrile in air (American Industrial Hygiene Association, 1970; Maddock et al., 1977). The sensitivity is in the range of 20-300 μg/ml of absorbing solution.

Another colorimetric method is based on the formation of cyanogen bromide under the influence of UVR and the production of a pink colour by coupling the cyanogen bromide with benzidine in pyridine solution. Using this method, Kanai & Hashimoto (1965) determined acrylonitrile in the expired air, blood, and urine of exposed animals. This method has been further used for the determination of acrylonitrile in air (Krynska, 1970; Tada, 1971; Russkih, 1972, 1973) with a detection limit of 0.4 - 0.5 mg/m^3, and in food (Kroeller, 1970) and waste water (Ghersin et al., 1969) with a detection limit of 2 mg/litre. When the sample contains both acrylonitrile and cyanide, the cyanide should be removed before analysis (Aldridge, 1944; Bruce et al., 1955; Kanai & Hashimoto, 1965).

(f) Titrimetric methods

A titrimetric method based on the cyanoethylation of a sulfhydryl compound (lauryl mercaptan), by acrylonitrile, has been described (Haslam & Newlands, 1955). An excess of the thiol is added to the acrylonitrile sample and, after the reaction, it is determined by iodometric or amperometric titration or by Ellman's reagent. Although this method is specific, it is neither rapid nor sensitive enough.

A titrimetric method for determining acrylonitrile, developed by Terent'ev & Obtemperanskaya (1956), consists of the release of sodium hydroxide by the reaction of acrylonitrile with sodium sulfite. A paper-strip modification of this method has recently been reported by Rajendran & Muthu (1981). It is used for the detection of acrylonitrile in air and fumigated foodstuffs.

(g) Other analytical methods

Other methods are not frequently used. The spectrophotometric method of Hall & Stevens (1977), in which formation of a pyridine-acrylonitrile complex is determined at 435.4 nm, suffers from interference from cyanide, which must be separated out of the solution.

2.2.2.1 Determination of acrylonitrile and its metabolites in biological materials

(a) Acrylonitrile in urine

Sato et al. (1975) have modified the method of Aldridge (1944); acrylonitrile in urine is separated by azeotropic distillation and then determined by gas chromatography. The detection limit is 5 µg/litre.

More recently, Houthuijs et al. (1982) developed a method using head-space chromatography, which has a detection limit of 2 µg/litre. Two millilitre aliquots of urine are equilibrated at 90 °C in 25 ml vials for 3-5 h; the vapour phase is injected automatically in a gas chromatograph, with a 15% carbowax column and a phosphor-nitrogen detector.

(b) Acrylonitrile metabolites in urine

(i) Acrylonitrile-derived mercapturic acids

A gas-chromatographic method has been developed by Draminski & Trojanowska (in press) for the determination of 2-cyanoethylmercapturic acid in the urine of workers exposed to acrylonitrile. The mercapturic acid is extracted from urine and derivatized by diazomethane to the methyl ester. The precision of this method is 10% for 2-cyanoethyl-mercapturic acid in the concentration range of 50-350 mg/litre of urine.

A general procedure for determining the total amount of mercapturic acids (more generally the total amount of thioethers) in urine has been described by Seutter-Berlage et al. (1977, 1978) and modified by Van Doorn et al. (1979) and Buffoni et al. (1982). Deproteinized urine is hydrolysed with sodium hydroxide for 50 min at 100 °C; this converts the mercapturic acids (and generally all thioethers) to the corresponding thiols. After cooling and acidification, SH-groups are assayed by the method of Ellman (1959).

(ii) Thiocyanate levels

The colorimetric method (the formation of a coloured complex of thiocyanate with ferric ion) was developed in 1943 (Lawton et al., 1943).

Very recently, Imanari et al. (1982) applied the high-performance liquid chromatographic technique, using a strong base ion-exchanger column for such determinations. The method has been shown to differentiate well between the urinary thiocyanate levels found in smokers and non-smokers.

3. SOURCES OF INDUSTRIAL AND ENVIRONMENTAL EXPOSURE TO ACRYLONITRILE

3.1 Natural Occurrence

Acrylonitrile does not occur as a natural product.

3.2 Industrial Technology, Production Data and Projection

Current production is based on catalytic ammoxidation of propylene in the vapour phase (Idol, 1974):

$$2CH_2 = CH-CH_3 + 2\ NH_3 + 3\ O_2 \xrightarrow{\text{catalyst}} 2\ CH_2 = CH-CN + 6\ H_2O$$

Bismuth phosphomolybdate is the most frequently used catalyst. The chief by-products are acetonitrile and hydrogen cyanide. Processes previously used in the production of acrylonitrile were: (i) the catalytic addition of hydrogen cyanide to acetylene; (ii) the catalytic dehydration of ethylene to cyanohydrin; and (iii) the catalytic reaction of propylene with nitric oxide. These processes are no longer used by the major manufacturers in the world.

In 1976, the known production of acrylonitrile was about 2.4 million tonnes (IARC, 1979). US manufacturers produced 0.69 million tonnes, Western European manufacturers, 0.92 million tonnes, and Japanese manufacturers, 0.63 million tonnes. Production figures for East European countries and the USSR are not available.

The average annual growth of acrylonitrile production was about 11% during 1965-1975 (Anonymous, 1977). While further growth was expected during the early 1980s, because of increased demands for polyacrylamide in tertiary oil recovery (Pujado et al., 1977), this did not occur owing to a general recession in world trade. The West European figure for 1981 is of the order of 800 000 tonnes (Personal communication), approximately 15% less than in 1976.

3.3 Use Patterns

The use pattern for acrylonitrile and its products in the USA in 1976 and Western Europe in 1977 are presented in Table 5 (IARC, 1979).

In a mixture with carbon tetrachloride, acrylonitrile has also been used as a fumigant for tobacco (Berg, ed., 1977) and for flour milling and bakery equipment.

Pesticides containing acrylonitrile have been withdrawn by the manufacturers. Acrylonitrile polymers and copolymers are

Table 5. The use patterns of acrylonitrile and its products in the USA (1976) and Western Europe (1977)[a]

Product	% of acrylonitrile production		% of product production
	USA	W. Europe	
acrylic and modacrylic fibres	48	68	82 - clothing and home furnishings 18 - export
acrylonitrile-butadiene-styrene and acrylonitrile-styrene resins	21	15	88 - pipe fittings, automotive vehicle components, etc. 12 - automobile instrument panels, household items etc.
adiponitrile	12	---	mainly hexamethylene-diamine
other products	19	17	21 - nitrile elastomers 21 - acrylamide 16 - barrier resins 42 - polyether polymer polyols, fatty diamines, etc.

[a] From: IARC (1979).

components of products intended for use in contact with food, e.g., (i) vinyl resin coatings; (ii) adhesives; (iii) cellophane; (iv) paper and paperboard components (limited); (v) polyolefin films; (vi) elastomers - for repeated use; and (vii) rigid, semi-rigid, and modified acrylic and vinyl plastics. In the USA, the amount of acrylic component may not exceed that which is reasonably required to produce the intended effect (US FDA, 1977b).

The acrylonitrile content of containers fabricated from acrylonitrile copolymers and the possible migration of acrylonitrile into foods and beverages have been reviewed (US FDA, 1977a). The use of copolymers of acrylonitrile for making beverage bottles was banned in the USA in September, 1977.

The Canada Food and Drugs Act and Regulations (1982) prohibit the sale of any food in packaging containing acrylonitrile, such that the compound may pass into the food.

Table 6 shows the levels of residual acrylonitrile in several polymers, some acrylonitrile derivatives, and products fumigated with acrylonitrile (US Consumer Product Safety Commission, 1978).

Table 6. Levels of residual acrylonitrile found in various products

Product	Acrylonitrile content
acrylic and modacrylic fibres	1 mg/kg[a]
acrylonitrile-butadiene-styrene resins	30-50 mg/kg[a]
styrene-acrylonitrile resins	15 mg/kg[a]
nitrile rubber and latex material	0-750 mg/kg[a]
acrylamide	25-50 mg/kg[a]
polyether polymer polyols	100-300 mg/kg[a]
shelled walnuts	0-8.5 mg/kg[b]
US cigarettes (non-filtered)	1-2 mg/100 cigarettes[c]

[a] From: US Consumer Product Safety Commission (1978).
[b] 38 days after fumigation with a mixture of acrylonitrile and carbon tetrachloride (Berck, 1960).
[c] From: Buérin et al. (1974); Wynder & Hoffmann (1967).

The total emissions from acrylonitrile plants in the USA, in 1974, have been estimated to be about 2.2% of the total production (Table 7) (Patterson et al., 1976). More recent estimates (Suta, personal communication, 1982), following the introduction of stricter emission controls, indicate an overall reduction in emissions and a change in pattern (for 1981, 800 tonnes for acrylonitrile production and 3000 tonnes for end-product manufacture).

3.4 Disposal of Wastes

Acrylonitrile may also enter the environment during storage, transport, transfer, and end-use. A detailed study on the entry of acrylonitrile into the environment was carried out by the Midwest Research Institute for the EPA (Going et al., 1979). Air, water, and soil were sampled at, and near to, acrylonitrile and acrylamide production plants and acrylonitrile-derived resin, fibre, and elastomer production plants.

During acrylonitrile production, the following wastes are produced: gaseous wastes; liquid wastes (waste water column bottoms, acetonitrile column bottoms, heavy ends, crude acetonitrile, hydrogen cyanide); and solid wastes (catalyst

Table 7. Acrylonitrile emissions from plants in the USA in 1974[a]

Source	Emission (tonnes)
acrylonitrile production	6400
end-product manufacture	5900
bulk storage	1800
total emission	14 100

[a] From: Patterson et al. (1976).

fines and organic polymers). Three types of on-site disposal methods have been described by Hughes & Horn (1977): (a) flare; (b) thermal incineration; and (c) deep-well pond and deep-well injection.

Much liquid waste from acrylonitrile-manufacturing plants is discharged directly into deep wells, after pre-treatment using alkaline hydrolysis, the biodegradable effluent being disposed of in publicly-owned treatment works. In some cases, organic wastes are incinerated (Lowenbach et al., 1978).

Deep-well injection is no longer considered a viable method in the USA; to control the drilling of new wells, an industrial discharger must re-apply for a permit (US EPA, 1977).

Lowenbach et al. (1978) extensively reviewed the alternative biological, chemical, and physical methods of treating waste waters from acrylonitrile manufacture, but a detailed discussion of these is not within the scope of this report.

3.5 Accidental Release

Acrylonitrile may be released accidentally into the environment. Its half-life in air is estimated to be 9-10 h (section 2.1.2). In water, the half-life, as estimated by the BOD test, is 5-7 days. Although these data would indicate that small spillages would not present a problem, initial high levels of acrylonitrile may have severe local effects. No bioaccumulation or food chain concentration potential has been noted (US Dept of Transportation, 1974), but it was observed that the concentration of acylonitrile in the ground water increased when it rained several months after an accidental spillage occurred. The persistence of acrylonitrile in the

water of wells located within 30 m of a spill of 91 000 litres of acrylonitrile from a tank car was followed for about 1 year (Miller & Villaume, 1978). No attempt was made to contain or clean up the spill for 108 days and water from 5 wells showed acrylonitrile concentrations ranging from 46 up to 3520 mg/litre during this time. On day 108, contaminated soil was removed, but levels of acrylonitrile actually increased in some wells. Levels decreased after about 170 days, when contaminated ground water was pumped away; a sample of this ground water contained an acrylonitrile concentration of 144 mg/litre. It is possible that the high concentration of acrylonitrile produced by the spill was lethal to bacteria, precluding biological degradation. However, no quantitative measurements of soil or water organisms were made.

3.6 Environmental Persistence

Acrylonitrile is readily degraded by acclimated anaerobic microorganisms (Mills & Stack, 1955). Aerobic degradation with activated sludge is complete in 20 days (Miller & Villaume, 1978; Freeman et al., 1981). The residual level after aerated activated sludge treatment was below 0.1 mg/kg. Acrylonitrile has been shown to inhibit anaerobic organisms (For fish toxicity, see Table 11, p. 58).

4. INDUSTRIAL AND ENVIRONMENTAL SOURCES AND LEVELS OF EXPOSURE

4.1 Exposure of the General Population

4.1.1 Air

The possibility of exposure to acrylonitrile-contaminated air is limited to residents near industrial production and processing sites. In the vicinity of 2 plants producing acrylonitrile, high concentrations of the monomer, ranging from 390 to 608 mg/m^3 (180-280 ppm) were found near the exhausts of both ships and storage tanks (Sato et al., 1979). Going et al. (1979) determined acrylonitrile concentrations in samples of air, soil, water, and sediments around 11 industrial sites. The concentrations of acrylonitrile in air varied from < 0.1 - 325 µg/m^3; the highest levels were found at an acrylonitrile-, butadiene-styrene resin plant and an acrylonitrile/acrylamide plant. The occurrence of acrylonitrile was highly correlated with the wind patterns; the highest levels were found downwind of the plant or at points crosswind but close to the plant. The air also contained xylenes, ethylbenzene, dichlorobenzenes, toluene, trimethylbenzenes, and styrene.

4.1.2 Water

Acrylonitrile was present in effluent discharged from chemical and latex manufacturing plants (Shackelford & Keith, 1976), and was detected at 0.1 g/litre in effluent discharged from an acrylic fibre-manufacturing plant in the USA (Europ-Cost, 1976). Near 11 industrial sites (Going et al., 1979), the highest acrylonitrile levels in water were 3.5 and 4.3 mg/litre from an acrylic/modacrylic fibre plant and a nitrile elastomer plant, respectively. There was no apparent correlation between air levels and water concentrations. No acrylonitrile was found in the soil and sediments. Water samples from some plants also contained propionitrile.

4.1.3 Food

Contamination of food from polymer packaging material containing free acrylonitrile has been reported. Following a study on the migration of acrylonitrile from ABS and AS resins, Tatsuno et al. (1979) concluded that after long-term preservation of food in ABS and AS resins the concentration of acrylonitrile in food may rise to 0.05 mg/kg. Further studies on food-simulating solvents showed that migration of

acrylonitrile occurred from ABS and AS resins into 4% acetic acid, 20% ethanol, heptane, and olive oil; it was concluded that resins containing acrylonitrile levels of more than 10 mg/kg should not be used for packaging foods containing alcohol (Tatsuno et al., 1980). Nitrile resins made from copolymers of acrylonitrile and other monomers (e.g., methyl acrylate) are no longer used in the USA to make beverage bottles (US FDA, 1977a). In a study performed in Sweden, the amount of acrylonitrile monomer found in nitrile resin bottles was 2-5 mg/kg. The amount in the beverage was generally 0.002 - 0.003 mg/kg, but two samples contained as much as 0.009 mg/kg (Vaz, 1981, personal communication). A government survey of the acrylonitrile content of food suggested that the average intake of acrylonitrile in the United Kingdom was likely to be less than 0.3 µg/person per day (United Kingdom Ministry of Agriculture, Fisheries & Food, 1982).

An acrylonitrile concentration of 0-19 mg/kg was detected in dry food fumigated with acrylonitrile at a concentration of 10 g/m^3. The study was carried out using radioactive acrylonitrile and provided information that acrylonitrile levels in the stored food decreased by 30-70% over a period of 2 months (Pfeilsticker et al., 1977).

4.1.4 Other sources of exposure

Free acrylonitrile monomer has been found in commercial acrylonitrile polymers at levels of less than 1 mg/kg (acrylic and modacrylic fibres), 15 mg/kg (styrene-acrylonitrile resins), 30-50 mg/kg (ABS resins) and 0-750 mg/kg (nitrile rubbers and latex materials) (US Consumer Product Safety Commission, 1978).

Another possible source of acrylonitrile environmental exposure is accidental spillage during transport. The following estimates have been made of the incidence of the accidental release of acrylonitrile per year: during transport in barges - 0.0117; in trucks - 0.063; and by rail - 0.17 (Miller & Villaume, 1978). This means, for example, that during transport by rail, one accident would occur approximately every 6 years.

4.2 Occupational Exposure

Up to 12 000 workers in the USA were thought to have come into major contact with acrylonitrile during 1976 and possibly some 125 000 workers were exposed, to some extent (Miller & Villaume, 1978). It has also been estimated that as many as 400 000 may have had some contact with acrylonitrile in 1976. The exposures reported in several countries are shown in Table 8.

Table 8. Concentration of acrylonitrile in the air at work-places

Operation	Acrylonitrile in work-place air (mg/m^3) Average level	Range	Reference
Acrylonitrile production	5 - 0.5[b]	-	Zotova (1975a)
during loading (open air)	5	0.2 - 60	Cincolella et al. (1981)
near ACN tanks or pumps	45	4 - 125	Cincolella et al. (1981)
	-	4.2 - 7.2	Gančeva et al. (1977)
Acrylic fibre production		3 - 20	Orusev et al. (1973)
	-	<11	Enikeeva et al. (1976)
	-	<11	Sakurai & Kusumoto (1972)
	-	<45	
	4.6 - 31[a,e]	<2.2 - 143	Sakurai et al. (1978)
	0.2 - 9.1[a,f]	-	
polymerization	8	<4 - 120	Czajkowska et al. (1969)
	<4	<1 - 110	Lodz Sanit. Inspec. Survey (1981)
	25	2 - 103	Cincolella et al. (1981)
spinning	6	1.5 - 20	Czajkowska et al. (1969)
	<4	<1 - 110	Lodz Sanit. Inspec. Survey (1981)
	9.5		Sakurai et al. (1978)

Table 8 (contd).

Thermosetting plastic plant	1.4		Scupakas (1968)
Rubber footwear plant		1 - 11	Volkova & Bagdinov (1969)
Unspecified chemical conversions	0.6 - 6		Babanov et al. (1959)
Production of acryl-butadiene-styrene resin (A.B.S)	4[a,s,t]	0 - 22	Iwasaki et al. (1980)
polymerization	30	0 - 200	Cincolella et al. (1981)
Production of nitrile rubber			
rubber - polymerization	4	1 - 27	Cincolella et al. (1981)
reactor cleaning	36[t]	5 - 54	Cincolella et al. (1981)
Acrylic dispersions (Latex production polymerization)	78	9 - 600	Cincolella et al. (1981)

[a] 2 or more factories evaluated.
[b] average levels over 5 years.
[t] time-weighted average concentration.
[s] spot.

The introduction of a lower exposure limit in several countries is likely to have decreased the actual exposure to acrylonitrile at the workplace.

As acrylonitrile vapour is twice as dense as air, spills and leaks in enclosed buildings may lead to harmful accumulations of vapour, especially in low-lying areas (Baxter, 1979). The same author describes various possibilities for preventing this, such as the use of double mechanical seals, enclosed drainage systems, well-ventilated sampling points, etc. Plant design should aim at complete containment of acrylonitrile, both as a liquid and a vapour.

A code of practice has recently been published for the safe design, construction, and use of plants (CIA, 1978). Safe handling, engineering, and work practices, controls, compliance programmes, personal protective equipment, housekeeping, employee information and training, signs and labels, etc. for work with acrylonitrile have been described by the OSHA (1981).

Exposure to acrylonitrile may also occur through skin contact. Acrylonitrile was shown to contaminate the skin of workers, their clothing and tools, also the equipment, walls, windows, handrails, handles, etc. in the workplace and was not easy to remove. A protective paste of household soap, mineral oil, glycerine, and china clay was said to reduce contamination of the palms of the hands by 67% (Zotova, 1975a).

Acrylonitrile can penetrate clothing and leather shoes (American Cyanamid, 1976). Dermal contact with liquid acrylonitrile may cause local skin damage, severe dermatitis, and systemic toxicity, and must therefore be prevented by high standards of industrial hygiene.

4.3 Estimate of Human Exposure from All Environmental Media

The production and use of acrylonitrile at the workplace provide the greatest potential for exposure. Airborne exposure to acrylonitrile near industrial sites appears to pose the highest potential risk for the general population; the potential for exposure through water and food appears to be low by comparison.

5. CHEMOBIOKINETICS AND METABOLISM

5.1 Absorption

5.1.1 Human studies

5.1.1.1 Uptake through inhalation

The retention of acrylonitrile in the respiratory tract in 3 volunteers exposed to a concentration of 20 mg/m^3 for up to 4 h was 46 ± 1.6% and did not change throughout the inhalation period (Rogaczewska & Piotrowski, 1968).

5.1.1.2 Dermal absorption

Rogaczewska & Piotrowski (1968) applied liquid acrylonitrile to the forearm skin of 4 human volunteers and estimated that the average absorption rate was 0.6 mg/cm^2 per h.

5.1.1.3 Uptake by other routes

No data available.

5.1.2 Experimental animal studies

5.1.2.1 Uptake through inhalation

Young et al. (1977) determined the recovery of ^{14}C acrylonitrile in rats exposed to 11 or 220 mg/m^3 (5 or 100 ppm) for 6 h in a "nose only" inhalation chamber. In the first 9 days following the start of inhalation, 82.2% of the radioactivity was recovered from the urine, after the higher dose, and 68.5%, after the lower dose, 3-4% occurred in the faeces; and 6% and 2.6%, respectively, were expired as $^{14}CO_2$.

5.1.2.2 Dermal absorption

Three rabbits breathing pure air while their skin (315-350 cm^2) was exposed to an atmosphere containing an acrylonitrile concentration of 1-4.2 g/m^3, survived, whereas 3 other rabbits breathing pure air with the skin exposed to 44-62 g/m^3 died within 2.5-4 h. Inhalation exposure to 0.58-0.67 g/m^3 was fatal for 3 rabbits within 2-3 h (Rogaczewska, 1975). The author interprets these data as suggesting that dermal absorption of vapour is about 100 times less efficient than its pulmonary absorption. The immersion

of rabbit ear in liquid acrylonitrile was fatal for the animal within a few hours (Rogaczewska, 1971).

Subcutaneous (sc) or intravenous (iv) administration of ^{14}C acrylonitrile at 0.5 mmole/kg body weight to rats resulted in faster and greater elimination of radioactivity in the first 4 h than after oral administration (Gut et al., 1980).

5.1.2.3 Uptake by other routes

Young et al. (1977) calculated that after oral administration of 0.1 mg or 10 mg of ^{14}C acrylonitrile per kg body weight, 85-100% of acrylonitrile was absorbed in rats. The absorption rate was lower in rats after oral administration than after sc or ip administration (Nerudova et al., 1980a; Gut et al., 1981). After ip administration, the blood concentration of acrylonitrile reached a maximum in several minutes and then decreased rapidly (Nerudova et al., 1980a; Gut et al., 1981). After ip and oral administration of 1,2-^{14}C acrylonitrile and acrylo-^{14}C-nitrile to rats, 82-93% of the radioactivity was recovered from the urine and some 3-7% exhaled unchanged in the breath in 24 h (Sapota, 1982).

5.2 Distribution and Toxicokinetics

5.2.1 Human studies

No data available.

5.2.2 Experimental animal studies

Acrylonitrile concentrations in blood and liver reach higher levels after iv or ip administration than after oral administration; concentrations rapidly decrease in blood ($t_{0.5}$ = 19 min) and liver ($t_{0.5}$ = 15 min after iv and 21 min after ip administration) (Nerudova et al., 1980a; Gut et al., 1981). The apparent $t_{0.5}$ after oral administration is 61 min in blood and 70 min in liver, but this appears to be due to slow absorption rather than to slow elimination. The area under the acrylonitrile concentration/time curve for blood was higher than for liver after oral, iv, or ip administration (Gut et al., 1981), indicating rapid transformation of acrylonitrile by the liver. Extrapolation of acrylonitrile blood levels after ip or iv administration in rats to zero time indicated that the apparent volume of distribution was unity, and that concentrations of free acrylonitrile in the rest of the body were unlikely to be greater than that in the blood (Nerudova et al., 1980a).

Young et al. (1977) followed the distribution of radioactivity in rats after a single oral or iv dose of ^{14}C acrylonitrile. Radioactivity was found in the lung, liver, kidney, stomach, intestines, skeletal muscle, blood, and other organs and tissues, but high levels of radioactivity occurred in erythrocytes, skin, and stomach regardless of the dose and route. The high levels in the stomach wall after iv administration support the observation of Nerudova et al. (1980a) that, after iv administration, acrylonitrile is excreted into the stomach lumen.

After single intraperitoneal and oral administration to rats of 1,2-^{14}C acrylonitrile and acrylo^{14}C-nitrile, most of the ^{14}C found in the tissues was associated with erythrocytes, liver, and kidneys, lower levels being found in the lung and brain. The ^{14}C in the erythrocytes was still largely retained 48 h after administration. Significant differences in the rates of ^{14}C loss from tissues occurred with 1,2-^{14}C acrylonitrile and acrylo^{14}C-nitrile given orally (Sapota & Draminski, 1981; Sapota, 1982).

After oral administration to rats, up to a maximum of 94% of ^{14}C from 1-^{14}C acrylonitrile in erythrocytes was found to be covalently bound to cytoplasmic and membrane proteins, whereas 90% of the radioactivity from potassium cyanide in erythrocytes was found in the haem fraction of haemoglobin (Farooqui & Ahmed, 1982).

After a single ip injection of 2,3-^{14}C acrylonitrile in male rats, radioactivity was generally highest in the blood, intermediate in the spleen, liver, and kidney, and lower in other tissues. The percentage of the dose remaining in the body after 9 days was estimated to be about 5% of the administered dose (Hashimoto & Kimura, 1977).

A semi-quantitative study using whole-body autoradiography (Sandberg & Slanina, 1980) confirmed that, after iv administration to rats, acrylonitrile and/or its metabolites accumulate in the blood, liver, kidney, stomach mucosa, adrenal cortex, intestinal contents, and hair follicles of rats. After oral administration to the monkey (Sandberg & Slanina, 1980), high radioactivity levels were detected in the liver, kidney, intestinal mucosa, adrenal cortex, and blood. As total radioactivity was measured in the studies of Young et al. (1977), Sandberg & Slanina (1980), and Sapota & Draminski (1981), it was impossible to differentiate acrylonitrile from its metabolites or from acrylonitrile bound covalently to proteins (Bolt et al., 1978; Gut et al., 1981); thus, these studies are difficult to interpret from the point of view of the chemobiokinetics of free acrylonitrile.

Peter & Bolt (1981) found that 12 h after ip or iv administration of 2,3-^{14}C acrylonitrile, about half of the radioactivity remaining in the tissues was irreversibly bound

to proteins. The rapid elimination of acrylonitrile mercapturic acid after iv, ip, or sc administration (Gut et al., 1981a) indicates that most of the acrylonitrile-derived radioactivity in the distribution studies was associated with cyanoethylglutathione, or subsequent intermediate metabolites including acrylonitrile mercapturic acid.

Thus, it is impossible to determine conclusively from the present data whether the relatively high levels of acrylonitrile-^{14}C radioactivity in the erythrocytes, kidney, spleen, liver, adrenals, stomach walls, and skin are due to free acrylonitrile, its metabolites, or cyanoethylated proteins. However, the chemobiokinetics of free acrylonitrile in blood and liver (Nerudova et al., 1981) suggest that its distribution is fairly uniform and that higher levels of radioactivity in some organs and erythrocytes are due to reaction products of acrylonitrile with soluble and protein sulfhydryls.

Information on the subcellular distribution of 1,^{14}C acrylonitrile in rat can be found in Ahmed et al. (1982). Sato et al. (1982) studied the distribution and accumulation of 2,3-^{14}C acrylonitrile in the rat. They observed a longer retention of acrylonitrile in brain and muscle. The cytosol fractions of brain, liver, and kidney showed a relatively high specific radioactivity.

The evidence, available at present, on the distribution of acrylonitrile in the body, and on tissue damage following exposure, does not indicate increased accumulation in any particular tissue or organ, except erythrocytes, and there is no indication from animal studies of tissue accumulation following long-term exposure.

5.3 Biotransformation and Elimination

Levels of acrylonitrile metabolites in blood and their relationship to atmospheric acrylonitrile concentrations or to the dose administered are usually considered together, in studies on the relationship between the dose or concentration of acrylonitrile and the elimination of metabolites in urine. They will therefore be considered together in the following section.

5.3.1 Human Studies

Acrylonitrile is metabolized partly to thiocyanate. Blood thiocyanate levels of volunteers exposed to acrylonitrile concentrations below 45mg/m^3 (22 ppm) for 30 min returned to normal after 24 h, while elevated levels were still present 12 h after exposure to 110 mg/m^3 (50 ppm) for 30 min (Wilson & McCormick, 1949).

Draminski & Trojanowska (in press) reported that at airborne acrylonitrile concentrations of between 3 and 10 mg/m^3, concentrations of S-(2-cyanoethyl) mercapturic acid in the urine of 13 workers exposed to acrylonitrile, fell in the range of 50-200 mg/litre,

5.3.2 Experimental animal studies

Acrylonitrile is partly metabolized to cyanide, which is then transformed by rhodanese (EC 2.8.1.1) to thiocyanate and eliminated in urine (Dudley & Neal, 1942; Brieger et al., 1952; Ghiringhelli, 1954). However, the fate of the major portion of administered acrylonitrile was not clear until recently. Recent studies have shown that the major urinary metabolites in rats, hamsters, guinea-pigs, rabbits, and dogs are mercapturic acids resulting from the glutathione-S-transferase(s) (EC 2.5.1.18) -catalysed conjugation of acrylonitrile or glycidonitrile with glutathione (section 5.3.2.2). At present, at least 10 acrylonitrile metabolites have been isolated and/or identified in animal urine.

The oxidative pathway leads to the liberation of cyanide via an epoxide (glycidonitrile) and cyanohydrin (Kopecky et al., 1980a,b). Cyanohydrin spontaneously decomposes to cyanide and glycolaldehyde which, together with 2-cyanoethanol, cyanoacetic acid, and acetic acid, have been found as in vitro metabolites of acrylonitrile (Duverger-van Bogaert et al., 1981). Only 2-cyanoethanol and cyanoacetic acid were detected in the urine of rats administered acrylonitrile intraperitoneally (Lambotte-Vandepaer et al., 1981a). The proposed routes of the oxidative pathway are shown diagrammatically in Fig. 1; some of the biotransformation steps are speculative.

The existence of a glucuronoconjugate of acrylonitrile was reported in the urine of rats after oral administration of acrylonitrile (Hoffman et al., 1976). Two metabolites of acrylonitrile (S-[2-cyanoethyl] cysteine and S-[2-cyanoethyl] mercapturic acid) were identified by Dahm (1977) in rats given radiolabelled acrylonitrile, but he was unable to identify a third metabolite, as it was unstable. Young et al. (1977) found that acrylamide was not a metabolite as had been suggested by Hashimoto & Kanai (1965). The same authors also identified carbon dioxide as a metabolite in rats, but they were unable to detect significant quantities of free acrylonitrile or cyanide in the urine of exposed rats, though Hashimoto & Kanai (1965) estimated that 15% of an iv dose of acrylonitrile was eliminated uncharged in the urine and expired air of the rabbit.

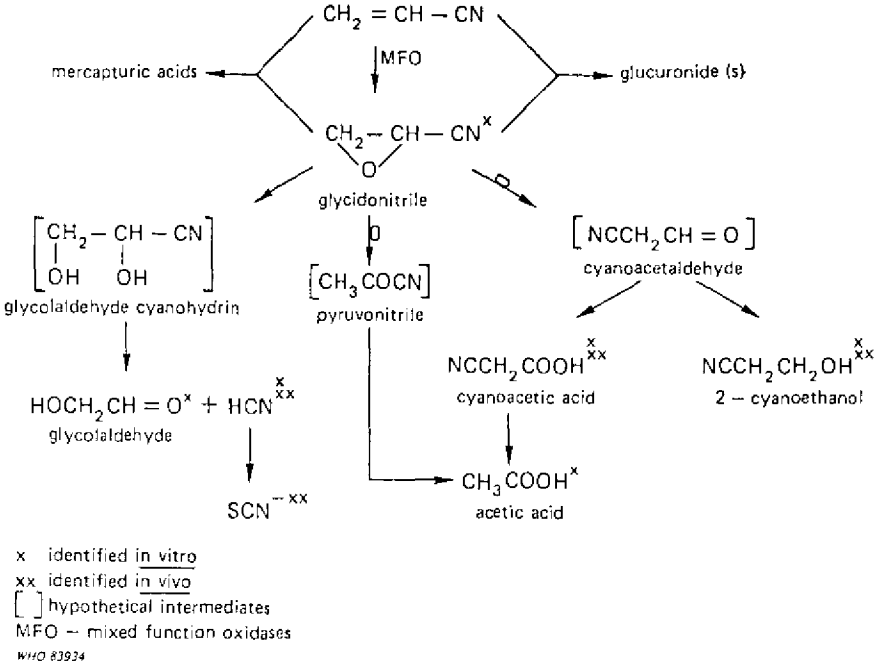


Fig. 1 The main possible pathways of acyrlonitrile biotransformation.

—□→ Chemical rearrangement.

5.3.2.1 The oxidative pathway of acrylonitrile metabolism

The oxidative pathway of acrylonitrile biotransformation includes a number of consecutive enzyme-catalyzed or spontaneous reactions. The first step, oxidation of acrylonitrile to glycidonitrile, is catalyzed by hepatic microsomal mono-oxygenases (Abreu & Ahmed 1980; Kopecký et al., 1980a,b; Guengerich et al., 1981; Ahmed & Abreu, 1982). Glycidonitrile is a reactive intermediate, and a number of its metabolites have been recorded; in in vitro experiments it is transformed by epoxide hydrolase (EC 3.3.2.3) to glycolaldehyde cyanohydrin, which decomposes spontaneously to hydrocyanic acid (cyanide) and glycoealdehyde (Kopecký et al., 1979, 1980a,b; Abreu & Ahmed, 1980; Duverger-van Bogaert, 1981a). The yield of cyanide in the in vitro experiments depends on the techniques used (Nerudova et al., 1980b). Besides forming conjugation products with glutathione (section 5.3.2.2), glycidonitrile rearranges to cyanoacetaldehyde, which is further reduced to 2-cyanoethanol or oxidized to cyanoacetic acid. Acetic acid is also present (Duverger-van Bogaert, 1981).

The results of animal studies have shown that cyanide formed in vivo is subsequently converted by rhodanese (EC 2.8.1.1) to thiocyanate and eliminated in urine (e.g., Dudley & Neal, 1942; Brieger et al., 1952; Ahmed & Patel, 1981). Thiocyanate has been directly measured in the urine of various animals after acrylonitrile administration (Lawton et al., 1943; Mallette, 1943; Czajkowska, 1971; Efremov, 1976b). Rats administered acrylonitrile at 60 mg/kg body weight, excreted thiocyanate in the urine at a constant rate of 0.53 mg/h with an excretion half period of 13 h (Czajkowska, 1971). Sulfhydryl compounds (cysteine, BAL, and Unithiol) increase the activity of rhodanese in the conversion of cyanide to thiocyanate in vitro, as well as in vivo (e.g., Frankenberg, 1980). A similar increase with acrylonitrile has not been convincingly demonstrated (Gut et al., 1975), perhaps because of the inhibiting properties of acrylonitrile on rhodanese.

5.3.2.2 Mercapturic acids formed in acrylonitrile biotransformation

Cyanoethylation of naturally-occurring sulfhydryl compounds plays an important role in acrylonitrile metabolism. Acrylonitrile forms stable conjugates with L-cysteine and L-glutathione in vitro (Hashimoto & Kanai, 1965; Gut et al., 1975) and a portion of absorbed acrylonitrile is thus prevented from being metabolized to cyanide. Depressed levels of sulfhydryl compounds have been reported following acrylonitrile administration (e.g.,

Wisniewska-Knypl et al., 1970; Hashimoto & Kanai, 1972; Vainio & Mäkinen, 1977; Dinu & Klein, 1976; Szabo et al., 1977). The spontaneous conjugation of glutathione with acrylonitrile or glycidonitrile proceeds very slowly; glycidonitrile forms S-(2-cyano-2-hydroxyethyl)-L-glutathione and S-(1-cyano-2-hydroxyethyl)-L-glutathione in the ratio of about 1:1. In the enzyme-catalysed conjugation this ratio shifts to about 3:1 (Holechek & Kopecký, 1981). These authors demonstrated that no cyanide was released from the conjugation product of acrylonitrile with GSH, while cyanide was released from the conjugation product of glycidonitrile with GSH. This study confirmed the findings of Boyland & Chasseaud (1967, 1968) concerning the participation of glutathione-S-alkylene transferase(s) (EC 2.5.2.18) in the cyanoethylation reaction of glutathione. Since glutathione conjugates are precursors of mercapturic acids, the occurrence of mercapturic acids derived from acrylonitrile and glycidonitrile may be expected in the urine of animals exposed to acrylonitrile.

The major metabolite of acrylonitrile in the rat, rabbit, and other animals was found to be 2-cyanoethylmercapturic acid (Dahm, 1977; Wright, 1977; Ahmed & Patel, 1979; Kopecký et al., 1979, 1980a,b,c, 1981; Langvardt et al., 1980; Sapota & Draminski, 1981; Sapota & Chmielnicka, 1981; Van Bladeren et al., 1981; Ghanayem & Ahmed, 1982). While 2-cyanoethylmercapturic acid was the sole mercapturic acid identified in the urine of rats after iv administration of acrylonitrile, a second mercapturic acid of unestablished structure was also excreted after oral administration. Langvardt et al. (1980), using 1-^{14}C- or 2,3-^{14}C-acrylonitrile, found seven radioactive metabolites in rat urine. The 3 major metabolites included thiocyanate and 2-cyanoethylmercapturic acid. The third was tentatively identified as 4-acetyl-5-cyanotetrahydro-1,4-2H-thiazine-3-carboxylic acid. The 4 remaining metabolites represented at least one third of the total activity excreted; their chemical structures are not known, but none contained the -CN group of acrylonitrile. Different results were reported by van Bladeren et al. (1981). In common with Kopecký & Langvardt and colleagues, they isolated 2-cyanoethylmercapturic acid from the urine of orally-dosed rats; however, 2-hydroxyethylmercapturic acid was also excreted. It is suggested that this second mercapturic acid may be formed via one of the conjugates of glutathione with glycidonitrile, S-(2-cyano-2-hydroxyethyl)-L-glutathione. The amount of mercapturic acids excreted relative to the dose was approximately constant up to a dose of acrylonitrile of 26.5 mg/kg body weight. At higher doses, the amount of mercapturic acids excreted remained constant. These authors and Wright (1977) suggested that this might be a consequence of the depletion of available glutathion at the higher dose levels.

It seems likely that, at high exposure levels, the preferred metabolic pathway (conjugation of glutathione with acrylonitrile or its metabolite) is overloaded, and another unknown metabolic pathway takes over. After an oral dose to rats of 1-^{14}C acrylonitrile, 4 metabolites were found in the bile, 2 major metabolites being GSH conjugates of acrylonitrile (Ghanayem & Ahmed, 1982).

The report by Dahm (1977) that rats administered acrylonitrile excreted S-(2-cyanoethyl)-L-cysteine has not been confirmed by any of the authors who have examined the glutathione conjugation pathway of acrylonitrile biotransformation. Fig. 2 illustrates the proposed routes of mercaptide formation from acrylonitrile.

5.3.2.3 The glucuronic acid conjugates of acrylonitrile metabolism

Rats treated with doses of acrylonitrile ranging from 20 to 40 mg/kg body weight (Hoffman et al., 1976) excreted significantly more glucuronic acid than untreated controls or rats administered 10 mg acrylonitrile/kg body weight. This suggests that acrylonitrile-derived glucuronide might be the alternative substance to conjugate metabolites (van Bladereu et al., 1981). The results of Lambotte-Vandepaer et al. (1980) support this theory. The mutagenicity of rat urine after administration of acrylonitrile at 30 mg/kg body weight was enhanced by treatment with β-D-glucuronidase (EC 3.2.1.31) prior to the Ames' mutagenicity assay. This indicates that a glucuronide was cleaved to give a free mutagenic agent derived from acrylonitrile. The dose fits the dose range that evokes a significant increase in glucuronic acid excretion (Hoffmann et al., 1976) and is of the same magnitude as that at which van Bladeren et al. (1981) demonstrated a depletion of glutathione in rat liver.

5.3.2.4 Quantitative aspects of acrylonitrile biotransformation and elimination of its metabolites

(a) Effect of acrylonitrile concentration and dose

The relationship between acrylonitrile concentrations in the air, cyanide and thiocyanate in the blood, and thiocyanate in the urine was described by Brieger et al. (1952). At acrylonitrile concentrations between 55 and 220 mg/m^3 (25 and 100 ppm), the blood and urine thiocyanate concentrations were proportional to inhaled acrylonitrile concentrations in rats. However, the cyanide content of blood was measurable only at the highest acrylonitrile concentration. In dogs, cyanide could be detected in blood at an acrylonitrile

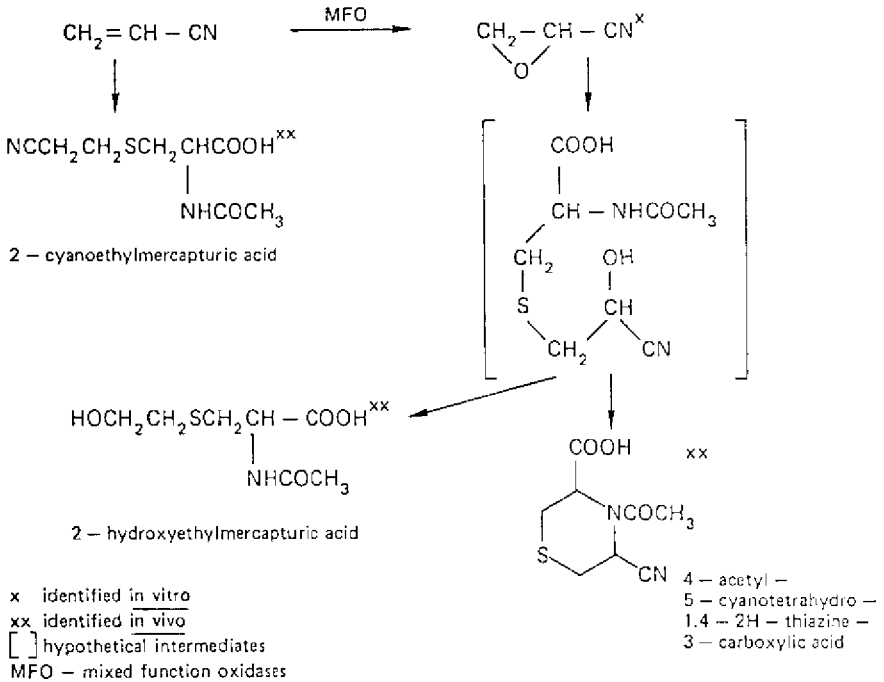


Fig. 2. The mercapturic acids derived from acrylonitrile and glycidonitrile and possible pathways.

concentration of 110 mg/m^3 and cyanide concentrations in blood were proportional to the inhaled acrylonitrile concentrations in the range of 110-220 mg/m^3 (50 - 100 ppm). Data indicate that a certain acrylonitrile concentration must be exceeded to provide conditions for the formation of enough cyanide to surpass the metabolic capacity of rhodanese or the supply of co-factors; this concentration is lower in the dog than in the rat.

In mice and rats, the dose of acrylonitrile was directly related to the cyanide levels in blood, liver, kidney, and brain (Ahmed & Patel, 1981), and, in rats, the ip administration of acrylonitrile at 20-60 mg/kg body weight or oral administration at 15-60 mg/kg body weight also produced a proportional increase in thiocyanate excretion in the urine.

However, thiocyanate is always present in urine (9 mg/litre in rats) (Brieger et al., 1952), and the acrylonitrile exposures required to exceed this level significantly are high. Thus, urine thiocyanate levels would not give an accurate estimate of exposure at the atmospheric acrylonitrile concentrations found in industry, at present.

The observation of Hoffmann et al. (1976) suggested a possible alternative conjugating route for metabolites at higher acrylonitrile exposure levels involving glucuronic acid. Before this is confirmed, the effects on carbohydrate metabolism and glucose utilization in rats must be considered, as well as the possibility that this alternative pathway of glucose metabolism leading to the formation of glucuronic acid, and thus elevated glucuronic acid levels in urine, may be stimulated by acrylonitrile. From the standpoint of a possible exposure test, however, it is emphasized that high doses of acrylonitrile are required to increase excretion of glucuronic acid in urine, but such doses would only occur in cases of accidental overexposure.

(b) Differences between species

The work of Brieger et al. (1952) revealed that, at the same acrylonitrile exposure concentrations, cyanide blood levels in dogs were far higher than in rats. This was apparently due to a less efficient detoxification of cyanide to thiocyanate in dogs since, when exposed to an acrylonitrile concentration of 217 mg/m^3 (100 ppm), the total sum of cyanide and thiocyanate concentrations in blood was about 260 µmol/litre in dogs and 840 µmol/litre in rats. Although the normal thiocyanate blood level was about 150 µmol/litre in the rat and only about 55 µmol/litre in the dog, the elevation caused by acrylonitrile was far higher in rats, suggesting that rats metabolize acrylonitrile to cyanide at a

substantially higher rate and are able to detoxify it more efficiently than dogs.

Mice excrete more thiocyanate than rats, at a given dose of acrylonitrile, even though detoxification of cyanide to thiocyanate in mice is apparently less efficient than in rats. Co-administration of acrylonitrile and thiosulfate resulted in a 3-fold increase in thiocyanate excretion in mice, while in rats the effect was much smaller (Gut et al. 1975; Silver et al., 1982). Moreover, the thiosulfate significantly reduced mortality in mice, but the reduction in rat mortality was only slight, confirming that enhanced detoxification of cyanide in mice is important.

Ahmed & Patel (1981) also observed that the rate of metabolism of acrylonitrile was higher in mice than in rats.

(c) Time course of elimination of acrylonitrile metabolites

The excretion in urine of ^{14}C-acrylonitrile-derived mercapturic acids follows shortly after ip, sc, iv, or oral administration of ^{14}C-acrylonitrile in rats (Gut et al., 1981a) and rapidly decreases, whereas the excretion of thiocyanate from acrylonitrile given orally or intraperitoneally increases after a time lag culminating between hours 8 and 12 in rats, but sooner in mice and Chinese hamsters (Gut et al., 1975). The time course of acrylonitrile-derived mercapturic acid excretion in rats was closely correlated with free acrylonitrile concentrations in blood and liver (Nerudova et al., 1980a; Gut et al., 1981a), while that of thiocyanate was not, whatever the route of administration.

(d) Effect of the route of administration

The excretion of thiocyanate by rats, mice, and Chinese hamsters after oral, ip, sc, and iv administration of ^{14}C-acrylonitrile represented 20-40%, 5%, 5%, and 1%, respectively, of the dose administered. However, urinary excretion of radioactivity was almost quantitative (Gut et al., 1981a); subtracting the thiocyanate excretion from total urinary metabolites (radioactivity) revealed that excretion of acrylonitrile-mercapturic acids (and other possible acrylonitrile metabolites) is independent of the route of administration (Kopecký et al., 1980a). When 1-^{14}C-acrylonitrile was administered orally to rats, 27% of the dose had been excreted in the bile in 6 h, mainly in the form of 2 glutathione conjugates of acrylonitrile (Ghanayem & Ahmed, 1982).

(e) Metabolic interactions of acrylonitrile with other xenobiotics

Oral administration of an equimolar dose of acrylonitrile (0.5 mmol/kg body weight) to rats did not influence the elimination of phenol from benzene. However, benzene, toluene, ethylbenzene, or styrene (0.5 mmol/kg body weight) markedly decreased the rate and total excretion of thiocyanate from an equal dose of acrylonitrile given orally; higher doses of the solvents caused greater inhibition (Gut et al., 1981). On the other hand, subcutaneous administration of benzene and styrene increased the excretion of an equal dose of ^{14}C-acrylonitrile (0.5 mmol/kg body weight) during the first 4 h and decreased it between the 8th and 12th hours (owing to inhibited thiocyanate formation and excretion). The total of metabolites excreted was unaffected. The co-administration of industrial solvents markedly increased the lethality of acrylonitrile (Gut et al., 1981a). Inhibition of the oxidative metabolism of acrylonitrile in rats by a cytochrome P-450 inhibitor (1-phenylimidazole) inhibited completely the excretion of N-acetyl-S-(2-hydroxyethyl) L-cysteine in favour of the excretion of N-acetyl-S-(2-cyanoethyl)-L-cysteine (van Bladeren et al., 1981). The latter compound, the authors considered, resulted from direct cyanoethylation of glutathione, whereas the former was formed via the epoxide, glycidonitrile. Overnight fasting and cobaltous chloride pre-treatment increased the biliary excretion of metabolites, while phenobarbital did not induce any change, and dimethyl maleate significantly decreased the excretion (Ghanayem & Ahmed, 1982).

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6. BIOLOGICAL MONITORING OF ACRYLONITRILE UPTAKE

Studies, particularly animal studies, on the absorption, distribution, biotransformation, and elimination of acrylonitrile have shown that a small fraction of the acrylonitrile absorbed is rapidly eliminated in the urine without biotransformation, while the remainder is biotransformed via several pathways, a number of metabolites being excreted in urine; some of these metabolites are unique to acrylonitrile.

The absorption studies have also clearly shown that, in addition to uptake of acrylonitrile by inhalation, skin penetration can be an important route of entry, particularly in the presence of liquid acrylonitrile. Thus, in human studies, unless performed under controlled conditions, a good correlation cannot necessarily be expected between a bioindicator of uptake and ambient air measurements of acrylonitrile, even when carried out with personal samplers.

Possible indicators of acrylonitrile uptake at present include: acrylonitrile in urine, acrylonitrile-derived mercapturic acids in urine, total thioethers in urine, and thiocyanates in urine.

Houthuijs et al. (1982) studied the excretion pattern of acrylonitrile in the urine of 15 exposed workers over a 7-day period, with a control group of 41 unexposed workers. They noted that the concentrations of acrylonitrile in urine peaked at the end, or shortly after the end, of the working day, decreasing rapidly until the beginning of the next working day without, however, falling to the levels in the control group. Correlations have been found between acrylonitrile concentrations in air and those in urine. In the control group, a significant increase in the acrylonitrile excretion in urine was found with the number of cigarettes smoked. For a mean acrylonitrile concentration in air of 0.28 mg/m^3 (0.13 ppm), the mean acrylonitrile level in urine at the end of the working day was 38 µg/litre, using the headspace chromatographic technique. In the control group for non-smokers, the mean level of acrylonitrile in urine was 2 µg/litre and for smokers (20-30 cigarettes per day) 9.0 µg/litre.

Sakurai et al. (1978) have also established a relationship between acrylonitrile concentrations in air and levels in urine for a group of 102 exposed workers and compared them with 62 controls. For an air concentration of 0.2 mg/m^3 (0.1 ppm) (as measured by personal samplers), an acrylonitrile level in urine of 3.0 µg/litre was found, using the Sato et al. (1975) method of analysis (separation by azeotropic distillation and determination by gas chromatography). The

determine the half-lives. This should make it possible to establish the most appropriate sampling time with respect to exposure and help in the determination of the concentrations of concern.

Interest in the determination of total "thioethers" in urine as a bioindicator of uptake lies in the greater simplicity of the analytical techniques used. However, more work is needed, particularly with regard to interferences and half-lives.

The possibility of estimating acrylonitrile exposure in smokers was suggested by Della Fiorentina & De Wiest (1979), who observed that determination of carboxyhaemoglobin in blood makes it possible to calculate the amount of thiocyanate present in urine that is due to smoking, and thus to calculate the uptake of acrylonitrile. However, experience shows that there can be marked variations in thiocyanate levels in smokers, which greatly exceed those in non-smokers occupationally-exposed to acrylonitrile (Czajkowska et al., 1969).

7. EFFECTS ON EXPERIMENTAL ANIMALS AND CELL SYSTEMS

7.1 Acute Toxicity

7.1.1 Lethal doses and concentrations

7.1.1.1 Lethal doses

The range of acute LD_{50} values for acrylonitrile in different laboratory mammals is generally between 25 and 186 mg/kg body weight (Table 9), though a value of 282 mg/kg body weight was observed when liquid acrylonitrile was applied to the skin of the tail of male rats (Zotova, 1976). Mice are more sensitive than rats, guinea-pigs, and rabbits. There seems to be little consistency in the effects of route or vehicle of administration, or of sex, on the LD_{50} level. The LD_{50} for dogs was not reported, but they tolerated iv administration of acrylonitrile at 50 mg/kg body weight and died after 300 mg/kg (Graham, 1965). The LD_{50} values reported are an order of magnitude higher than the LD_{50} for cyanide (one of the metabolites of acrylonitrile), but markedly lower than those for industrial solvents and monomers of plastics (the LD_{50} for benzene and its derivatives being about 2000-3000 mg/kg body weight).

7.1.1.2 Lethal concentrations in the air

The range of acute LC_{50}s for 4-h inhalation of acrylonitrile is between 150 and 1250 mg/m^3 (Table 10). Dogs appeared to be the most sensitive of the species tested and the sensitivity decreased in the following order: mice, rabbits, cats, rats, guinea-pigs, the latter being apparently the most resistant to inhalation exposure. The exposure of 315-350 cm^2 of the skin of rabbits to an acrylonitrile concentration of 44-62 g/m^3, in an exposure chamber, such that the animals were breathing pure air, proved fatal after 2.5-4 h. Inhalation exposure to 0.58-0.67 g/m^3 was fatal for 3 rabbits within 2-3 h (Rogaczewska, 1975).

In the 3 species of insects tested in a fumigation chamber for 8 h, the LC_{50} value was found to be 700-1900 mg/m^3 (Bond & Buckland, 1976). Lindgren et al. (1954) exposed 8 insect species for 2 or 6 h and found LC_{50} values of 1000-4500 mg/m^3.

Table 9. Acute LD_{50} values for acrylonitrile: effect of animal species strain and route of administration

Species/strain/sex	Number	Route	LD_{50} (mg/kg body weight)	Vehicle	Reference
mouse/-/male	M + F 333	oral	36	water	Tullar (1947)
mouse/-/female	M + F 333	oral	48	water	Tullar (1947)
mouse/-/M + F	169	oral	40	olive oil	Tullar (1947)
mouse/H strain/-	-	oral	25	physiol. saline	Beneš & Černa (1959)
mouse/-/-	-	oral	40-46	-	American Cyanamid (1951)
mouse/-/female	M + F 325	ip	48	water	Tullar (1947)
mouse/-/male	M + F 325	ip	40	water	Tullar (1947)
mouse/NMRI or "SPF"/-	-	ip	50	-	Zeller et al. (1969)
mouse/ICR/female	-	ip	47	-	Yoshikawa (1968)
mouse/H strain/-	-	sc	35("technical AN")	physiol. saline	Beneš & Černa (1959)
mouse/"inbred"/male	60	sc	50 (2 h) 25 (24 h)	physiol. saline	Graham (1965)
mouse/BN/male	60	sc	34	-	Knobloch et al. (1971)
rat/Sherman/-	groups of 6-10	oral	93	-	Smyth & Carpenter (1948)
rat/Wistar/-	-	oral	101	-	Paulet & Vidal (1975)
rat/Wistar or Stock/-	-	oral	128	-	Zeller et al. (1969)
rat/Wistar-Stamm/male	-	oral	82	-	von Borchardt et al. (1970)
rat/Wistar-Stamm/female	-	oral	86	-	von Borchardt et al. (1970)
rat/-/M + F	80	oral	84	water	Tullar (1947)
rat/-/M + F	51	oral	72	olive oil	Tullar (1947)
rat/Wistar/-	-	oral	78	physiol. saline	Beneš & Černa (1959)
rat/Sprague-Dawley/male	20	oral	186	water	Monsanto (1975)
rat/Sprague-Dawley/female	20	oral	186	water	Monsanto (1975)

Table 9 (contd).

rat/Wistar/male	110	ip	100	-	Knobloch et al. (1971)
rat/Wistar/-	-	ip	65	polyethylene glycol	Paulet & Vidal (1975)
rat/Wistar/male	110	sc	80	-	Knobloch et al. (1971)
rat/"albino"/male	-	sc	96	water	Magos (1962)
rat/"white"/male	-	skin of tail	282	liquid acrylonitrile	Zotova (1976)
rat/"white"/male	-	skin of abdomen	148	liquid acrylonitrile	Zotova (1976)
guinea-pig/-/-	-	oral	50	-	Carpenter et al. (1949)
guinea-pig/-/-	-	oral	85	olive oil	Tullar (1947)
guinea-pig/-/M & F	30	oral	56	-	Jedlicka et al. (1958)
guinea-pig/-/-	-	sc	130	-	Ghiringhelli (1954)
guinea-pig/-/-	11	iv	72	water	Tullar (1947)
guinea-pig/Hartley-/male	12 or more	intact skin	0.46 ml/kg	-	Roudabush et al. (1965)
		abraded skin	0.86 ml/kg	-	Roudabush et al. (1965)
guinea-pig/-/-	-	skin	0.25 ml/kg	-	Smyth & Carpenter (1948)
rabbit/-/-	-	oral	93	-	Lefaux (1966)
rabbit/-/-	-	iv	69	-	Paulet & Desmos (1961)
rabbit/"white"/M & F	12 or more	abraded skin	0.28 ml/kg	-	Roudabush et al. (1965)

Table 10. Acute lethal effect of single inhalation of acrylonitrile: effect of duration and concentration of acrylonitrile

Species/strain/sex	Number	Concentration (mg/m^3)	Duration (h)	Mortality (died/tested)	Reference
white mouse/stock/-	6	600	0.5	0/6	McOmie (1949)
	6	1500	0.5	5/6	McOmie (1949)
	6	5800	0.5	5/6	McOmie (1949)
	6	900	1	1/6	McOmie (1949)
	6	900	2	3/6	McOmie (1949)
	6	1700	1	6/6	McOmie (1949)
mouse/BN/male	12	300	4	LC_{50}	Knobloch et al. (1971)
rat/Sherman/-	6	1085	4	0/6	Smyth & Carpenter et al. (1971)
	6	2170	4	6/6	Smyth & Carpenter et al. (1971)
rat/Sherman/female	6	1085	4	2/6 to 4/6	Carpenter et al. (1949)
rat/Wistar/-	20	54	7	0/20	Brieger et al. (1952)
	20	109	7	0/20	Brieger et al. (1952)
	20	163	7	0/20	Brieger et al. (1952)
	20	217	7	4/20	Brieger et al. (1952)
rat/Wistar/male	12	470	4	LC_{50}	Knobloch et al. (1971)
rat/Osborne-Mendel/-	16	2750	1	0/16	Dudley & Neal (1942)
	16	3230	1	4/16	Dudley & Neal (1942)
	16	5300	1	13/16	Dudley & Neal (1942)
	16	660	2	0/16	Dudley & Neal (1942)
	16	1290	2	6/16	Dudley & Neal (1942)
	16	2730	2	16/16	Dudley & Neal (1942)
	16	280	4	0/16	Dudley & Neal (1942)
	16	680	4	5/16	Dudley & Neal (1942)
	16	1380	4	16/16	Dudley & Neal (1942)
	16	290	8	0/16	Dudley & Neal (1942)

Table 10 (contd).

rat/Osborne-Mendel/- (contd)	16	460	8	1/16	Dudley & Neal (1942)
	16	590	8	7/16	Dudley & Neal (1942)
	16	690	8	15/16	Dudley & Neal (1942)
rat/Wistar/male	3	650	3	1/3	Appel et al. (1981)
	3	1100	2	3/3	Appel et al. (1981)
	3	2600	0.5	1/3	Appel et al. (1981)
	6	3000	0.5	6/6	Appel et al. (1981)
guinea-pig/-/-	8	580	4	0/8	Dudley & Neal (1942)
	8	1250	4	5/8	Dudley & Neal (1942)
	8	2520	4	8/8	Dudley & Neal (1942)
guinea-pig/-/-	12	990	4	LC_{50}	Knobloch et al. (1971)
rabbit/"albino"/-	2	290	4	0/2	Dudley & Neal (1942)
	2	560	4	2/2	Dudley & Neal (1942)
	2	1260	4	2/2	Dudley & Neal (1942)
rabbit/-/-	5	670 - 1100	2-3	5/5	Rogaczewska (1975)
cat/-/-	4	210	4	0/4	Dudley & Neal (1942)
	2	600	4	0/2	Dudley & Neal (1942)
	2	1300	4	2/2	Dudley & Neal (1942)
dog/-/-	3	63	4	0/3	Dudley & Neal (1942)
	2	140	4	1/2	Dudley & Neal (1942)
	3	213	4	0/3	Dudley & Neal (1942)
	2	240	4	2/2	Dudley & Neal (1942)
dog/-/-	4	108	7	0/4	Brieger et al. (1952)
	4	163	7	0/4	Brieger et al. (1952)
	6	213	7	6/6	Brieger et al. (1952)
Rhesus monkey/-/-	3	163	7	1/3	Brieger et al. (1952)

7.1.1.3 Lethal concentrations in water

(a) Fish

Acute toxicity, determined by a static bioassay at 25 °C, revealed TL_m (median tolerance limit, i.e., a concentration of acrylonitrile killing 50% of the test organisms within a specified time) values ranging from 25.5 to 44.6 mg/litre at 24 h, and from 11.8 to 33.5 mg/litre at 96 h. There were no apparent significant differences in the sensitivity of various kinds of fish (Table 11).

(b) Invertebrates

For the brown shrimp (Crangon crangon), the LC_{50} for a 24-h exposure was 10-33 mg/litre (Portman & Wilson, 1971). Bandt (1953) exposed several species of arthropods (a shrimp-like crustaceae and 3 types of larvae) to 20-100 mg acrylonitrile/litre water and found marked species and individual differences: a lethal effect was observed in some species with 25 mg/litre after 48 h, while other species were not affected after 3 days. The most resistant species were unaffected by 100 mg/litre after 24-48 h. The results of studies by Rajendran & Muthu (1981) showed that acrylonitrile affects the activity of the phosphorylase and acetylcholinesterase enzymes in Tribolium castaneum Herbst, and Trogoderma granarium Everts.

7.1.2 Clinical observations

The inhalation studies of Dudley & Neal (1942), Brieger et al. (1952), and Rogaczewska (1975), and the results of oral and parenteral administration (Ghiringhelli, 1954; Benesh & Cherna, 1959; Paulet & Despos, 1961; Graham, 1965; Paulet et al., 1966) indicate that animals inhaling lethal concentrations of acrylonitrile, or administered lethal dosages of acrylonitrile orally or parenterally, showed rather similar effects: excitability and stimulated breathing, shallow rapid breathing, slow gasping breathing, apnoea, convulsions, and death. Vomiting occurred in cats, dogs, and monkeys after inhaling acrylonitrile, and in rats following parenteral administration. Reddening of the skin of the ears, nose, and feet (in rhesus monkeys, also of the face and genital organs) and mucosa was accompanied by lachrymation, nasal discharge, and salivation, not only after inhalation exposure, but also following oral and sc administration, while hind-leg incoordination, paresis or paralysis, were observed in rats after oral administration, and in rabbits after iv administration.

Table 11. Median tolerance limit values (TL_m)[a] for various fish exposed to acrylonitrile

Species	Water type	TL_m (mg/litre) 24 h	48 h	96 h	Reference
Fathead minnow (Pimphales promelas)	hard	32.7	16.7	14.3	Henderson et al. (1961)
	soft	34.3	21.5	18.1	Henderson et al. (1961)
Minnow (Phoxinus phoxinus)	-	38.2	17.6	-	Marcoci & Ionescu (1974)
Bluegill (Lepomis macrochirus)	soft	25.5	14.3	11.8	Henderson et al. (1961)
Guppy (Lebistes reticulatus)	soft	44.6	33.5	33.5	Henderson et al. (1961)
Goldfish (Carassius sp.)	-	-	-	40	Paulet & Vidal (1975)
Carp (Cyprinus carpio)	-	37.4	24.0	-	Marcoci & Ionescu (1974)
Rainbow trout (Salmo gairdneri)	hard	-	70	-	Jackson & Brown (1970)
Pin perch (marine fish) (Lagodon rhomboides)	sea (30/1 tank)	24.5	-	-	Daugherty & Garett (1951)
Rainbow trout (Salmo gairdneri)	tap, dechlorinated, 3.6 mg/litre	-	5[b]	-	Sloof (1979)
	hard	15	-	-	Sloof (1979)
Zebra fish	same	(LC_{50})			

[a] TL_m median tolerance limit, a concentration of acrylonitrile killing 50% of the test organisms within a specified time.

[b] Minimal concentration changing respiratory frequency.

(a) Effects on the skin

Direct application of liquid acrylonitrile to the shaved skin of rabbits induced slight local vasodilation immediately, without any systemic effect (1-2 ml covering 100-200 cm^2) or with an increased respiratory rate (3 ml over 300 cm^2) (McOmie, 1949). Tuller (1947) observed erythema in only one of 3 areas of abraded skin, following application of 1 ml of acrylonitrile on a gauze pad covered by rubber sheeting. However, Zeller et al. (1969) found that a 15-min application of acrylonitrile on a cotton pad to shaved skin resulted in skin oedema, and a 20-h application, in slight necrosis. Guinea-pigs appear to be more sensitive than rabbits; the application of a 2% solution of acrylonitrile in acetone for 24 h, under occlusion, did not induce any effects, but 8% or higher concentrations induced dose-dependent erythema followed by desquamation and mild necrosis (Gut et al., unpublished data). Erythema of the nose, face, ears, legs, and genital organs may follow inhalation and oral administration of acrylonitrile.

(b) Effects on the eye

McOmie (1949) instilled one drop of acrylonitrile into the eye of a rabbit. After 1 h, there was mild conjunctivitis without corneal clouding or pupillary damage and no effects were observed after 24 h. Oedema and slight necrosis of the conjunctiva after 8 days were observed in rabbits by Zeller et al. (1969).

(c) Effects on respiration

It was stressed by Paulet et al. (1966) that, after a lethal intravenous dose of acrylonitrile (120 mg/kg body weight), the respiratory rate in rabbits did not increase as is characteristic in cyanide poisoning. However, respiratory disturbance was observed in: guinea-pigs given a sc lethal dose of acrylonitrile (130 mg/kg body weight) (Ghiringhelli, 1954), anaesthetized dogs given 100 mg/kg body weight intravenously (Graham, 1965), mice given an oral lethal dose (Benesh & Cherna, 1959), and in guinea-pigs given 100 mg/kg body weight orally (Jedlicka et al., 1958). Pulmonary oedema was also seen.

An increased respiratory rate followed the application of liquid acrylonitrile (3 ml/kg body weight) to the skin of rabbits (McOmie, 1949), and "respiratory distress" was reported in rhesus monkeys exposed to 163 mg/m^3 for 7 h (Brieger et al., 1952). When rats, rabbits, cats, dogs, and monkeys were exposed to lethal concentrations of acrylo-

nitrile, Dudley & Neal (1942) observed an initial stimulation of respiration followed by shallow rapid breathing, slow gasping breathing, convulsions, coma, and death. These respiratory effects were absent in guinea-pigs, but irritation of the pulmonary membranes and some delayed deaths from lung oedema occurred.

(d) Effects on circulation

Acrylonitrile administered iv at 13, 27, 55, or 110 mg/kg body weight had little effect on the respiratory and blood pressure responses of anaesthetized rabbits to adrenalin, noradrenalin, or acetylcholine, and Gravczyk & Zwierzchovski (1973) believed that the circulation was not the primary target organ in acrylonitrile poisoning. However, lethal doses (50 or 100 mg/kg body weight) in guinea-pigs caused dilation of the right ventricle, congestion of the coronary blood vessels, hepatic and splenic hyperaemia, and inflammation of the intestinal mucosa (Jedlicka et al., 1958). In Sprague-Dawley rats administered a lethal dose of acrylonitrile, there were haemorrhagic areas in the lung and liver and acute gastrointestinal inflammation (Monsanto, 1975). Whether the reddening of nose, ears, legs, face, and genital organs in rats and other species, after inhalation and oral administration of acrylonitrile, is due to a direct effect on small vessels or is an inflammatory response is not known.

(e) Effects on adrenals

The effect of lethal doses of acrylonitrile on the adrenals became evident in the reports of Szabo & Selye (1971, 1972) and Szabo et al. (1976). After iv administration of high doses (150 or 200 mg/kg body weight), haemorrhage was observed in both adrenals of most animals, and there was adrenal haemorrhage in some rats following oral administration of 10, 15, or 20 mg/kg body weight. Various types of histological damage were observed in the adrenal cortex and medulla, some of them within 30 min of acrylonitrile administration.

A possible mechanism involving the peroxidative action of acrylonitrile in acrylonitrile-induced adrenal injury has been suggested recently by Silver & Szabo (1982). Szabo et al. (1980) investigated the pathogenesis of experimental adrenal haemorrhagic necrosis using various morphological, biochemical, and pharmacological methods. Their results suggest that the presence of a functional adrenocortex is necessary for the development of cortical damage.

(f) Blood chemistry

Intraperitoneal administration of acrylonitrile to male rats at 33 mg/kg body weight per day for 3 days decreased serum corticosterone to 30%, prolactin to 40%, but increased follicle-stimulating hormone (FSH) to 200% of control levels and did not affect luteinizing hormone (LH) (Nilsen et al., 1980). In adult male Wistar rats, a single ip administration of acrylonitrile of 10 mg/kg body weight did not have any effect on serum glutamic oxaloacetic transferase (SGOT) and serum glutamic pyruvate transaminase (SGPT) activity, but increased lactate dehydrogenase (EC 1.1.1.27) (LDH) to 200% and sorbitol dehydrogenase (SDH) (EC 1.1.1.14) to 300% compared with the controls (Noel et al., 1978). The same dose in male rats inhibited butyrylcholineesterase (EC 3.1.1.8), did not have any effect on alkaline phosphatase (EC 3.1.3.1), and increased fructose monophosphate aldolase activity, suggesting that there had been an adverse effect on the liver (Ivanov et al., 1979). Administraton of L-cysteine, alpha tocopherol, or ionol prevented these effects. A single oral dose of acrylonitrile (1/2 LD_{50}, 41 mg/kg body weight) in rats resulted in changes in the elution patterns of serum gel chromatography and paper-electrophoresis of globulins (Franzen & Wagner, 1978). Serum SDH was significantly elevated (approximately 4-fold) in rats, 24 h after administration of acrylonitrile at 150 mg/kg body weight. A 60% increase in serum SDH was found in rats administered acrylonitrile at 500 mg/litre in drinking-water for 21 days (Silver et al., 1982).

(g) Effects on other organs

Focal superficial necrosis of the liver associated with haemorrhagic gastritis was found in rats necropsied 24 h after administration of acrylonitrile at 150 mg/kg body weight in the drinking-water (Silver et al., 1982).

Acrylonitrile shows an inhibitory effect on K-stimulated respiration of guinea-pig brain cortex slices at 1 mM, but little effect on the liver at the same concentration. A stronger anaesthetic action of acrylonitrile was detected *in vitro* on the sciatic nerve of *Rana nigra maculata*, compared with some other anaesthetic agents (Hashimoto & Kanai, 1965). The recovery phase of nerve excitation was also affected by acrylonitrile (Ando & Hashimoto, 1967).

7.1.3 Biochemical changes and mechanisms of acrylonitrile toxicity

7.1.3.1 Effect on cytochrome oxidase

Evidence has been presented that cytochrome oxidase (EC 1.9.3.1) activity may be significantly inhibited in acrylonitrile poisoning. This was suspected after Dudley & Neal (1942) and Brieger et al. (1952) had reported significant concentrations of cyanide in dogs and monkeys exposed to acrylonitrile vapours. Tarkowski (1968) observed inhibited cytochrome oxidase activity in the brain, kidneys, and liver of rats after ip injection of acrylonitrile at 100 mg/kg body weight. In vitro, a 50% inhibition of the enzyme was observed in homogenates of brain, kidneys, and liver with an acrylonitrile concentration of 10^{-3}M. Such concentrations have been observed in vivo, shortly after lethal doses of acrylonitrile (Tarkowski, 1968; Nerudova et al., 1980; Gut et al., 1981b), but acrylonitrile has a short half-life in blood and liver after ip or iv administration (15-20 min). There are correspondingly increased blood and liver concentrations of cyanide in rats after acrylonitrile administration (up to 180 µM, Gut et al., 1981b), together with an even greater inhibition of cytochrome oxidase, and a much higher sensitivity of cytochrome oxidase activity to cyanide (50% inhibition in vitro at 10^{-8}M (Tarkowski, 1966).

A significantly decreased ratio of oxidized to reduced nicotinamide-adenine dinucleotides was observed by Sokal et al. (1972, 1977) in the brain of rats after sc administration of acrylonitrile at 100-120 mg/kg body weight, indicating inhibition of NADH oxidation in the mitochondria, possibly also at the level of cytochrome oxidase. These changes seem to be of biological importance, because their magnitude was similar to that observed at the death of animals subjected to experimental hypoxia. Thus, the cyanide-mediated inhibition of cytochrome oxidase would seem to be of importance in the later stages of intoxication and death. This "cyanide effect" is apparently more pronounced at higher acrylonitrile doses (Willhite & Smith, 1981) and appears to be more significant in mice and dogs (Brieger et al., 1952; Benesh & Cherna, 1959; Gut et al., 1981b) than in rats. This is in agreement with the greater efficacy in mice than in rats of thiosulfate (an antidote to cyanide) in acrylonitrile poisoning, and with the higher cyanide concentration in the blood of dogs than in that of rats (101 µM versus 10 µM), after breathing the same concentration of acrylonitrile (217 mg/m^3 for 7 h) (Brieger et al., 1952). The protective effect of another cyanide antidote, nitrite (Dudley & Neal, 1942; Ghiringhelli, 1954·

Benesh & Cherna, 1959), in acrylonitrile poisoning also points to the participation of cyanide in lethal acrylonitrile poisoning.

7.1.3.2 Effect on sulfhydryls

There is considerable evidence to demonstrate that acrylonitrile significantly depresses the concentrations of soluble glutathione and protein sulfhydryls in the blood, liver, brain, and kidney. Acrylonitrile also inhibits some SH-dependent enzymes that participate in carbohydrate metabolism. Wisniewska-Knypl (1970, 1978), Hashimoto & Kanai (1972), and Vainio & Mäkinen (1977) observed that the inhibition of sulfhydryls was dose-dependent in the range of 10-100 mg/kg body weight *in vivo*, and in the concentraton range 0.01-10 nM *in vitro* (Wisniewska-Knypl, 1978). A significant decrease in brain sulfhydryls was reported after a single dermal application of acrylonitrile of as little as 2.82 mg/kg body weight (Zotova, 1976). These effects were observed after sc, ip, or iv administration to rats, rabbits, hamsters, guinea-pigs, and mice. There were some decreases in the activity of serum or tissue - SH-dependent enzymes including oxoglutarate dehydrogenase (EC 1.2.4.2). However, the activity of succinate dehydrogenase (EC 1.3.99.1) was not reduced (Wisniewska-Knypl, 1978), and there were corresponding increases in the liver, blood, and brain concentrations of glucose, pyruvate, and lactate (Hashimoto & Ando, 1966; Dinu & Klein, 1976).

It was shown by Zitting et al. (1981) that short-term exposure to acrylonitrile decreased the liver glutathione content within 4 h of poisoning, but that the glutathione contents returned to normal in brain, liver, and kidney, within 24 h. At the same time, the activity of cerebral succinate dehydrogenase and of ethoxycoumarin demethylase in liver and kidney decreased. Increased glucose, pyruvate, and lactate concentrations in blood, liver, and brain were also found, immediately after the fifth exposure, in rats exposed through inhalation to an acrylonitrile concentration of 300 mg/m^3, 8 h daily, for 5 days. In protein, sulfhydryl-dependent enzyme inhibition was absent and the glutathione level was significantly reduced in the liver but not in the brain (Gut et al., 1982). The effects of acrylonitrile on sulfhydryls were significantly reduced by co-administering L-cysteine and other sulfhydryls and there were corresponding decreases in lethal effects (Hashimoto & Kanai, 1965; Bondarev et al., 1976; McLaughlin et al., 1976; Appel et al., 1981). The results of these studies demonstrate the protective role of SH-groups in acrylonitrile poisoning.

The role of hypoxia in the acute thiol-depressive effect of acrylonitrile in male rats was investigated by Jaeger (1978) and Jaeger & Cote (1982). Hypoxia was found to enhance non-protein SH loss in the liver, when there was exposure to acrylonitrile (Jaeger & Cote, 1982).

Evidence has been presented (Holechek & Kopecký, 1981) that inhibition of tissue sulfhydryls may be due not only to the acrylonitrile itself, but also to its reactive metabolite, glycidonitrile.

7.1.3.3 Interaction with the microsomal oxidation system as a possible mechanism of toxicity

Acrylonitrile added to mouse, rat, and human liver microsomes caused characteristic spectral complexes with cytochrome P-450 (Ivanov et al., 1979; Appel et al., 1981).

It was shown in vitro that glycidonitrile, which is generated in rat-liver microsomes by mixed-function oxidases (EC 1.14.14.1), covalently binds to microsomal membrane and albumin (Ivanov et al., 1982). The biological significance of this phenomenon may be inferred from experiments with inhibitors and inducers of the mixed-function oxidases (Ivanov, 1981). In rats pre-treated with phenobarbital, an increased amount of glycidonitrile was covalently bound to macromolecules and substantially higher activity of fructose-bisphosphate aldolase (EC 4.1.2.13) was observed in the blood, indicating liver damage. On the other hand, SKF-525A, the inhibitor of cytochrome P-450, reduced both effects in vivo and in vitro. Activation of acrylonitrile by cytochrome P-450 may therefore result in a cytotoxic effect.

A previous injection of SKF-525A or of cobalt(II) chloride ($CoCl_2$), another inhibitor of cytochrome P-450, resulted in significant protection against the gastrointestinal bleeding in rats caused by acrylonitrile (Ghanayem & Ahmed, 1982).

Some data show that acrylonitrile and its epoxide, glycidonitrile, bind covalently, in vitro, to DNA and RNA (Guengenrich et al., 1981; Peter et al., 1983a). However, the quantitative extent of truly irreversible binding is much less than that observed in experiments with other vinyl monomers (Peter et al., 1983).

A lower content of cytochrome P-450 in the liver and reduced oxidative microsomal metabolism of xenobiotics were observed in rats after an ip injection of acrylonitrile at 10 or 33 mg/kg body weight, for 3 days (Noel et al., 1978; Nilsen et al., 1980), after inhalation exposure to 300 mg/m^3 of acrylonitrile, 8 h day, for 5 days (Gut et al., in press), and in Chinese hamsters after an ip injection of 30 mg/kg (Zitting

et al., 1981). Inhibition of microsomal oxidation of xenobiotics by acrylonitrile was observed in vitro (Ivanov et al., 1979).

Pre-treatment of rats with inducers of microsomal oxidases such as phenobarbital, 3-methylcholantrene, or Arochlor 1254, nullified the effect of acrylonitrile on the total cytochrome P-450 content. The activity of other microsomal enzymes, glucose-6-phosphatase (EC 3.1.3.9), and NADPH cytochrome-c-reductase (EC 1.6.2.4), was unaffected by acrylonitrile (Duverger-van Bogaert et al., 1978; Noel et al., 1978).

Ghanayem & Ahmed (1982) showed that Arochlor 1254 drastically increased acrylonitrile-induced gastric bleeding in rats. Phenobarbital significantly increased acrylonitrile-induced hepatocyte damage in rats (Ivanov, 1981).

Acrylonitrile binds with liver microsomal and S-9 fractions, in vitro, by direct alkylation. The microsomal activation of acrylonitrile into reactive intermediates was also detected (Duverger-van Bogaert et al., 1982a). The irreversible binding of acrylonitrile to liver microsomal proteins was inhibited by thiols and even more by dithiocarb (Peter & Bolt, 1981).

7.1.3.4 Observations on the possible participation of membrane lipid peroxidation in the mechanism of toxicity

The ip administration of acrylonitrile at 10 mg/kg body weight induced lipid peroxidation in rat liver (Dinu, 1975a; Ivanov et al., 1979) and erythrocyte membranes (Ivanov et al., 1982), indicating possible damage of cellular membranes. NADPH-dependent lipid peroxidation in rat liver microsomes was only slightly stimulated (Ivanov et al., 1978; Duverger-van Bogaert et al., 1981) whereas substantial stimulation was found in the post-mitochondrial fraction of rat liver, lung, and brain, as well as in brain-marrow homogenate (Ivanov et al., 1978; Ivanov, 1979; Al'shansky et al., 1980). There was a correlation between an increased amount of malondialdehyde and a decreased content of -SH groups in the post-mitochondrial supernatant of rat liver (Ivanov, 1981) and brain (Al'shansky et al., 1980). Conjugated diene concentrations in rat liver microsomes were significantly elevated after iv administration of acrylonitrile to rats (150 mg/kg body weight), but no change was seen in the adrenal glands (Silver & Szabo, 1982).

Pre-treatment of rats with antioxidants, in doses equivalent to those of the acrylonitrile administered, afforded protection against the pro-oxidant effect of acrylonitrile and elevation of blood fructose-1-phosphate aldolase, decreased the activity of butyrylcholine esterase (EC 3.1.1.8) (Ivanov et al., 1979), and reduced the GABA

content and activity of glutamate decarboxylase (EC 4.1.1.15) in the brain (Al'shansky et al., 1980).

7.1.3.5 Studies on antidotes

Appel et al. (1981a) found that the cyanide antidotes, 4-dimethylaminophenol plus thiosulfate, protected rats against the lethal effects of orally-administered acrylonitrile. A comparative evaluation was made by McLaughlin et al. (1975) of the efficacy of thiols (cysteine hydrochloride) and cyanide antidotes. The authors showed that thiols were more effective in protecting rats against acrylonitrile poisoning. Bondarev et al. (1976) demonstrated the protective role of some sulfur-containing compounds in the acrylonitrile poisoning of rats. The protective effects of some antioxidants, such as vitamin E and ionol, have also been demonstrated (Ivanov et al., 1979).

The possible toxic mechanisms and theoretical protective mechanisms are summarized in Fig. 3, which indicates the complex nature of the interference of acrylonitrile with cellular mechanisms, as far as can be derived from current knowledge.

7.2 Subacute Toxicity

7.2.1 Inhalation exposure

Rats, guinea-pigs, rabbits, monkeys, and cats were exposed to acrylonitrile at 330 mg/m^3 air for 4 h per day, 5 days a week, for 8 weeks. All adult rats survived 8 weeks, but 5 out of 8 young rats died by the 6th week, 3 out of 16 guinea-pigs and 1 out of 4 rabbits died during the 5th week, and 1 out of 2 monkeys died after 6 weeks of exposure. At 220 mg/m^3 for 4 h/day, 5 days a week, for 8 weeks, all rats, rabbits, and guinea-pigs survived for 8 weeks, but 1 out of 4 cats died during the third week. After the first 4-h exposure to 120 mg/m^3, 1 out of 2 dogs died, but the other survived 4 weeks' exposure. Four rhesus monkeys survived 4 weeks' exposure to 120 mg/m^3 for 4 h/day (Dudley et al., 1942).

CD-1 mice, Charles River rats, and beagle dogs were exposed to acrylonitrile 57 times for 6 h a day, 5 days a week, over a 90-day period. Some dogs were killed by exposure to 117 mg/m^3 (54 ppm) but not 58 mg/m^3 (24 ppm). Mice and rats were unaffected by these concentrations, but a concentration of 234 mg/m^3 (108 ppm) was lethal for half the rats and mice. As with acute exposures, dogs were more sensitive than rats and mice, but mice did not appear to be more sensitive than rats. Atmospheric concentrations of

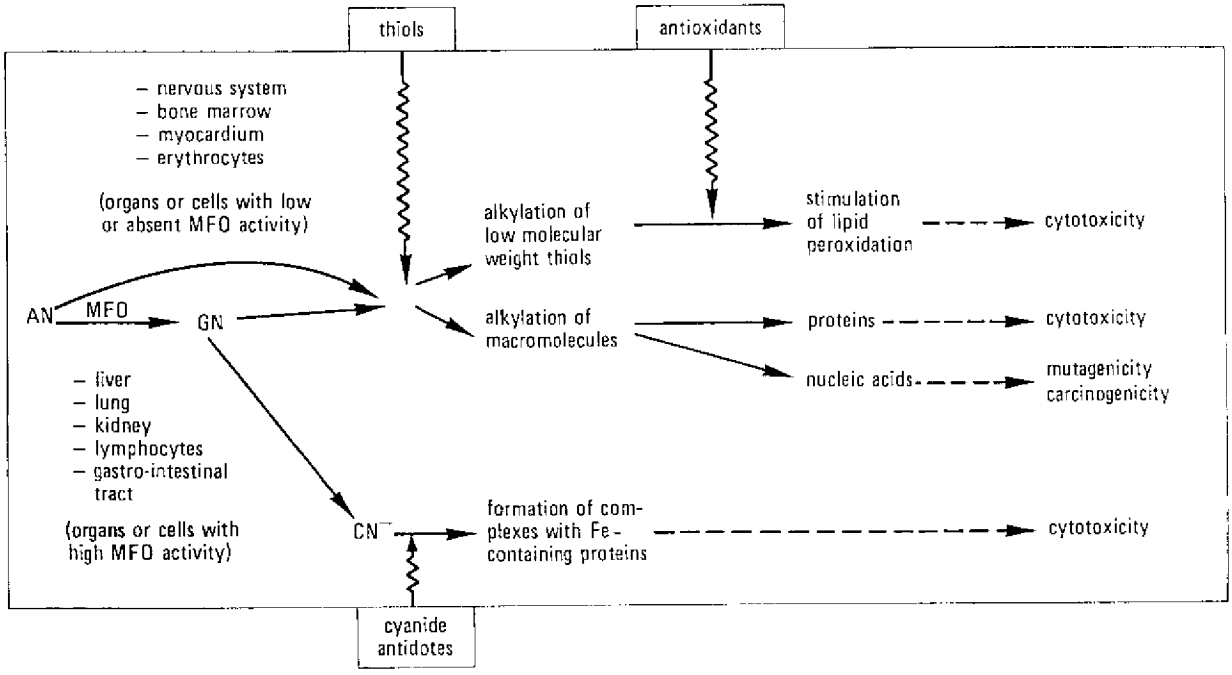

Fig. 3. Possible mechanisms of acrylonitrile toxicity and theoretical chemical protective mechanisms.

⟶ Possible ultimate effect.
⟶ Direct effect.

acrylonitrile of 58, 117, and 117 mg/m^3 did not induce any lethal effects in dogs, rats, and mice, respectively (Brewer, 1976).

7.2.2 Oral administration

Over a period of 7 weeks, 6 rats were administered orally 15 doses of acrylonitrile at 30 mg/kg body weight, then 7 doses at 50 mg/kg, followed by 13 doses at 75 mg/kg, without lethal effects being induced (Barnes, 1970). No deaths occurred when Sprague-Dawley rats were offered 85 mg or less of acrylonitrile per litre of drinking-water for 90 days (Humiston & Frauson, 1975). Given the slow absorption of acrylonitrile from the gastrointestinal tract, blood levels could have been low, and it can be calculated that the daily dose could have been about 8.5 mg/kg body weight. The studies are compatible with the view that acrylonitrile is unlikely to have a significant cumulative effect.

7.2.3 Subcutaneous administration and intraperitoneal administration

Daily sc doses of 40 mg/kg body weight over 4 weeks, or daily ip injections of 20 mg acrylonitrile/kg body weight over 6 weeks, were not fatal for rats (Krysiak & Knobloch, 1971).

7.2.4 Clinical observations in animal studies

Rats exposed to acrylonitrile concentrations of 220 mg/m^3 for 4 h daily, 5 days a week (Dudley et al., 1942) over a period of 8 weeks, showed slight lethargy but gained weight, as did guinea-pigs. Rabbits failed to gain weight and were listless, while cats became listless, vomited, and lost weight. One cat developed a transitory weakness of the hind legs after the third exposure and died after the eleventh exposure; the 3 remaining cats survived 8 weeks with few untoward effects. Exposure to an atmospheric concentration of 330 mg/m^3 resulted in weight loss in rats, their coats became rough, and their general physical condition poor (Dudley et al., 1942). Young rats and guinea-pigs showed impaired growth and marked irritation of the eyes and nose during the first week of exposure. Marked eye and nose irritation was also seen in rabbits and cats and the latter developed transitory weakness of the hind legs. Monkeys appeared sleepy and weak and frequently salivated and vomited. Thus, the 220 mg/m^3 exposure level markedly affected cats, while rabbits and guinea-pigs were little affected; a concentration of 330 mg/m^3 induced various effects including death. Brewer (1976) exposed CD-1 mice,

Charles River rats, and beagle dogs to acrylonitrile concentrations of 0, 58, 117, 234 mg/m^3 (0, 24, 54, or 108 ppm) for 6 h daily, 5 days a week, for 13 weeks. Signs observed included ataxia, ptosis, emaciation, rhinitis, and diuresis. As is common with acrylonitrile over-exposure, convulsions usually preceded death (e.g., Benesh & Cherna, 1959; Paulet et al., 1966).

7.2.4.1 Body weight, food and water consumption

Loss of body weight or failure to gain body weight was seen in rats exposed to acrylonitrile at 330 mg/m^3 and in cats and rabbits exposed to 220 mg/m^3 for 4 h a day, 5 days a week, for 13 weeks. Loss of appetite was seen in rhesus monkeys exposed to 330 mg/m^3, while 120 mg/m^3 did not elicit any toxic effects (Dudley et al., 1942). No adverse effects were seen in 6 rats administered successively 15 doses of 30 mg/kg body weight, 7 doses of 50 mg/kg, and 13 doses of 75 mg/kg over a period of 7 weeks (Barnes, 1970). Adult Sprague-Dawley rats received between 0 and 42 mg acrylonitrile/kg body weight in drinking-water for 90 days. The body weight was depressed in males receiving 42 mg/kg, while females were affected by 22 mg/kg, but only after the 57th day. The mean weekly food consumption of males was lower for 7 weeks on 38 mg/kg and for 2 weeks on 17 mg/kg. Food consumption decreased in females receiving 42 or 22 mg/kg for 6 weeks and 1 week, respectively (Humiston & Frauson, 1975).

7.2.4.2 Organ weights and pathology

The weights of the liver, kidney, spleen, pituitary gland, lungs, gonads, thyroid gland, adrenals, heart, and brain of rats, mice, and dogs exposed to an acrylonitrile concentration of 234 mg/m^3 for 6 h a day, 5 days a week, for 13 weeks, were within normal limits (Brewer, 1976). In rats receiving acrylonitrile in drinking-water for 90 days, no changes in absolute or relative organ weights were seen in males receiving 4 mg/kg body weight or females receiving 5 mg/kg, daily (Humiston & Frauson, 1975). The relative liver weight was significantly increased in males and females receiving 17 mg/kg (males) or 22 mg/kg (females) or more. The weights of the heart and liver were increased significantly in adult Wistar rats administered 50 mg acrylonitrile/kg body weight, intraperitoneally, daily, for 3 weeks (Knobloch et al., 1971); their weight loss caused an increase in the relative weights not only of the heart and liver, but also of the kidney and spleen.

Dudley et al. (1942) examined the livers of rats, guinea-pigs, rabbits, cats, dogs, and rhesus monkeys exposed to

acrylonitrile at 220 or 230 mg/m^3, and observed histological changes only in cats. Liver parenchymal degeneration was reported in adult Wistar rats after daily ip administration of 50 mg/kg body weight for 3 weeks (Knobloch et al., 1971). In the above-mentioned study, Dudley et al. (1942) also reported signs of renal damage, such as hyaline casts in the straight collecting tubules of all species, and limited subacute interstitial nephritis; this was especially seen in guinea-pigs and rabbits. Parenchymal degeneration of the kidneys was reported by Knobloch et al. (1971). In the study by Dudley et al. (1942), lungs were affected by subacute bronchopneumonia, congestion, and oedema of the alveolar walls, extravasation of erythrocytes and serum into the alveoli, focal collection of lymphyocytes and polymorphonuclear leukocytes, in most guinea-pigs, rabbits, the monkey, and 1 out of 3 of the rats. The authors also reported slight haemosiderosis in the spleen of rats, but negligible siderosis in cats, guinea-pigs, and rabbits. Exposure of rats to acrylonitrile (22 mg/m^3, 10 ppm) for 7 weeks and (100 mg/m^3, 50 ppm) for another 6 weeks caused enlargement of the liver, kidney, heart, and spleen, but co-administration of vitamins B1, B2, and cystine had a protective effect against enlargement of the heart. Alcohol dehydrogenase activity in the liver decreased after exposure, but the above-mentioned drugs alleviated this decrease, to some extent (Takagi et al., 1968).

7.2.4.3 Blood

A normal haematological picture was reported in rats and dogs repeatedly exposed to acrylonitrile vapours at up to 240 mg/m^3 (Brewer, 1976), and in rats and rabbits (except for a raised eosinophil count) repeatedly exposed to up to 330 mg/m^3 (Dudley et al., 1942).

Minami et al. (1973) exposed male rabbits to 54 mg/m^3 for 1 day per week (8 h) for 8 weeks; haematocrit and haemoglobin were unaffected, but pO_2 and pH were raised and pCO_2 lowered by the treatment.

In rats receiving acrylonitrile in the drinking-water for 90 days, the only significant haematological change was a decrease in the red-cell count, on the 83rd day, in females receiving 42 mg/kg body weight per day (Humiston & Frauson, 1975). The blood urea and alkaline phosphatase (EC 3.1.3.1) levels in the males receiving 38 mg/kg body weight per day were raised, but SGPT activity was normal. While rats administered ip 50 mg acrylonitrile/kg body weight, daily, for 3 weeks (Knobloch et al., 1971) developed leukocytosis and increased serum asparagine-oxo-aminotransferase (EC 2.6.1.14) activity, mice exposed for 70 days to 225 mg/m^3 (100 ppm) or

340 mg/m^3 (150 ppm) 6 h daily, did not develop any haematological abnormalities (Hashimoto, 1962). Rats exposed to 9.7 mg/m^3, 4 h daily, 5 days a week for 2 months, did not show any effects on the erythrocyte count or on the haemoglobin concentration (Vissarionova et al., 1979).

As a whole, the studies failed to demonstrate any consistent effect of acrylonitrile on red or white blood cell production or viability. Leukocytosis following repeated intraperitoneal administration of an irritant material is to be expected.

7.2.4.4 Immune system

Wistar rats were exposed to an acrylonitrile concentration of 10 mg/m^3 for 6 h daily, 5 days a week, for 16 weeks. Acrylonitrile depressed both T helper and T suppressor functions, and a diminished degree of B lymphocyte transformation was observed. Alpha tocopherol (im 0.21 mmol/kg body weight on alternate days for 16 weeks) protected against this effect (Krivova et al., 1982).

7.2.4.5 Nervous system

Some findings in the experimental animal studies on acrylonitrile were indicative of an effect on the nervous system. Rats, mice, and dogs exposed to up to 240 mg/m^3, for 6 h daily, 5 days per week, for 13 weeks, showed ataxia and convulsions, prior to death (Brewer, 1976). Transitory hind-leg weakness was seen in cats exposed for 8 weeks (4 h per day, 5 days per week) to 330 mg/m^3 (Dudley et al., 1942). Krysiak & Knobloch (1971) found that rats receiving acrylonitrile intraperitoneally at 20 mg/kg body weight daily for 6 weeks, or sc at 40 mg/kg daily for 4 weeks, showed a significant lengthening of the time to perform correctly in a conditioned food reflex test, and a significant decrease in the number of correct reactions, compared with pre-treatment observations or controls. Performance improved when the treatment was discontinued. Daily ip administration to rats of acrylonitrile at 50 mg/kg body weight for 3 weeks caused a vacuolization of neuronal cells of the cortex and brain stem (Knobloch et al., 1971).

7.2.4.6 Urine

No significant changes in urine composition were observed in the experimental studies of Humiston & Frauson (1975) (sections 7.2.4.1 - 7.2.4.3).

7.2.4.7 Adrenals

The adrenals of rats exposed for 21-60 days to acrylonitrile in drinking-water (0.05% and 0.2%) showed an atrophic zona fasciculata and an enlarged zona glomerulosa. The animals on the higher dose had a reduced plasma corticosteroid level and an increased plasma Na^+ concentration. The K^+ level was unchanged (Szabo et al., 1976). Serum corticosterone in rats was decreased by 3 ip doses of acrylonitrile (33 mg/kg body weight) on successive days (Nilsen et al., 1980).

7.2.4.8 Metabolism

After repeated exposure of rabbits to acrylonitrile, the in vitro metabolism in the liver of acrylonitrile into cyanide and thiocyanate decreased with time, while the excretion of unchanged acrylonitrile in the urine increased (Sato, 1978).

7.3 Chronic Toxicity

Observations have been made on animals administered acrylonitrile in drinking-water, food, through inhalation, and by dermal application.

Tuller (1947) administered acrylonitrile at 500 mg/litre in the drinking-water or acrylonitrile-fumigated food (dose not precisely specified) to rats. After 2 years, the mortality was higher in rats drinking acrylonitrile solution (50% deaths) than in paired controls (25%), another control group (15%), and in rats on acrylonitrile-fumigated food (5%). However, when acrylonitrile was administered at 0.5, 5, and 90 mg/litre in the drinking-water to male and female CFW rats for 2 years, the mortality rate was unaffected (Svirbely & Floyd, 1961). Groups of 4 male and 4 female beagle dogs were administered acrylonitrile at concentrations of 100, 200, or 300 mg/litre in the drinking-water, for 6 months. Average intakes of acrylonitrile were the following for males (females): 10(8) mg/kg body weight at 100 mg/litre; 16(17) mg/kg at 200 mg/litre; and 17(18) mg/kg at 300 mg/litre. Five dogs died, or were killed because debilitated, in each of the 2 higher dosage groups (Quast et al., 1975).

In the dogs receiving acrylonitrile at 100-300 mg/litre in the drinking-water, early signs of toxicity included roughening of the coat and, later, retching and vomiting. Terminal signs of lethargy, weakness, emaciation, and respiratory distress were noted (Quast et al., 1975).

7.3.1 Body weight, food and water intake

Body-weight gain was reduced during 4 of the 11 weeks at the higher dose level (240 mg/m^3) in a test in which male and female Wistar rats and albino rabbits were exposed to acrylonitrile vapour at concentrations of 0, 50, and 240 mg/m^3, for 3 h/day, 6 days a week, for 6 months (Knobloch et al., 1972).

Growth retardation was observed in male rats drinking 500 mg acrylonitrile per litre water, for 2 years (Tuller, 1947). Svirbely & Floyd (1961) administered acrylonitrile at 0.5, 5, or 50 mg/litre in the drinking-water of rats and found a slight decrease in water consumption at the highest concentration in both sexes. Statistically significant reductions in the body weight of rats receiving drinking-water containing acrylonitrile concentrations of 35, 100, or 300 mg/litre were associated with decreased water consumption and decreased food consumption at 300 mg/litre in males and 100 mg/litre in females (Quast et al., 1977). Decreased food and water consumption and body weight was reported with beagle dogs drinking acrylonitrile at 200 or 300 mg/litre water for 6 months (Quast et al., 1975). Marked weight decreases were seen in dogs that eventually died or had to be killed.

7.3.2 Organ weights

In rabbits exposed to atmospheric acrylonitrile concentrations of 250 mg/m^3, for 3 h/day, 6 days a week for 6 months, a significant increase in the heart weight was noted, and some fluctuations in blood pressure were described (Knobloch et al., 1972).

Ferin et al. (1961) exposed rats to drinking-water containing an acrylonitrile concentration of 20 or 1000 mg/litre for 6 months. At the higher dose, increased relative weights of the liver, spleen, and kidneys were noted. The relative weights of the heart, liver, and brain in males, and of the liver and kidneys in females, were increased in rats receiving 300 mg/litre in drinking-water. The males drinking 100 mg/litre showed a significantly increased brain weight, and the females receiving 100 mg/litre had significantly lower relative heart weights (Quast et al., 1977). The weights of brain, heart, liver, and kidneys of beagle dogs drinking an acrylonitrile concentration of 100 mg/litre in drinking-water (Quast et al., 1977) were normal but, at 200 mg/litre, the 2 remaining males had a lower absolute brain weight and higher relative kidney weight than controls. The 2 remaining females receiving 300 mg/litre also had signifantly lower relative brain weights compared with controls.

7.3.3 Pathology and histology

Inflammation of the pulmonary system accompanied by an inflammatory exudate into the bronchial lumen occurred in rats exposed to acrylonitrile at 240 mg/m^3 for 3 h a day, 6 days a week, for 6 months (Knobloch et al., 1972). Various pathological changes occurred in male and female rats maintained on water containing acrylonitrile at 35, 100, or 300 mg/litre for 12 months (Quast et al., 1977). Males and females on the 2 higher doses developed paleness and thickening of the mucosa, erosions, ulcers, and sometimes papilloma formations in the non-glandular portion of the stomach. Three females receiving 300 and 100 mg/litre and one male at 300 mg/litre had ear canal tumours. Microscopic findings of tissue with tumorous growth revealed an increased frequency of gastric cell papillomas, Zymbal (sebaceous) gland tumours of the ear canal, and microtumours of the nervous system, in rats receiving a concentration of acrylonitrile of 100 or 300 mg/litre. These tumours do not occur spontaneously at such a high frequency in the strain of rat used. The nervous system lesions were consistent with the diagnosis of astrocytoma.

Minimal lesions were seen in the liver of rats drinking acrylonitrile at 100 or 300 mg/litre and chronic renal disease occurred in females drinking 300 mg/litre. The squamous epithelium of the stomach was hyperplastic in rats drinking 100 or 300 mg/litre.

Histopathological changes in dogs receiving acrylonitrile concentrations of 200 and 300 mg/litre in water (Quast et al., 1975) were similar to those in untreated controls. The pneumonia present may have resulted from irritation of the mucosa of the tongue and oesophagus, which produced abnormal swallowing, resulting in aspiration of some food.

7.3.4 Haematology and clinical chemistry

In rats exposed for 3 h/day, 6 days a week, for 6 months to acrylonitrile in air concentrations of either 50 or 240 mg/m^3, the eosinophil count had significantly increased after 4 months. Total serum protein was unchanged, while albumin and alpha-globulin had increased and gamma-globulin decreased in both test groups (Knobloch et al., 1971). Leukocytosis was observed in rats drinking acrylonitrile in water at 1000 mg/litre (Ferin et al., 1961). Periodic examinations of rats drinking 0.5, 5, or 50 mg/litre in water (Svirbely & Floyd, 1961), or 0 - 300 mg/litre in water (Quast et al., 1977), showed normal haematological findings. A significant elevation in alkaline phosphatase activity was found in female rats exposed to 300 mg/litre. Half-way

through the study, beagle dogs drinking acrylonitrile concentrations of 0, 100, 200, or 300 mg/litre in water showed a significant decrease in haematocrit, erythrocyte count, and haemoglobin concentration at 300 mg/litre and decreased erythrocyte count at 200 mg/litre, in males. Females on 300 mg/litre also showed a significant decrease in the erythrocyte count. However, all haematological findings were within normal ranges towards the end of the study, except in males at 300 mg/litre, which had a lower erythrocyte count. Blood urea nitrogen, serum alkaline phosphatase activity, SGPT, and SGOT were measured periodically; the findings in males were always within normal limits, but females receiving 300 and 200 mg/litre showed some increase in SGOT and SPGT activity. Total and individual serum proteins were unaffected at the end of the study. Zotova (1976) exposed rats by applying acrylonitrile solution to the skin of the tail at doses of 2.82, 0.56, or 0.11 mg/kg body weight per day and observed a decreased haemoglobin concentration at the highest dosage level after 2 months. Blood catalase (EC 1.11.1.6) activity increased, and blood peroxidase (EC 1.11.1.7) activity decreased, initially, but later became normal; there was no change in sulfhydryl levels.

A decrease in blood -SH groups was reported in rats exposed to acrylonitrile at 10 mg/m^3 for 5 days/week, 4 h a day, for 4 months (Efremov, 1976d).

7.3.5 Nervous system

Changes in central nervous function, as elicited by a conditioned avoidance test, were found in rats drinking water containing acrylonitrile at 20 mg/litre, for 6 months (Ferin et al., 1961). Rats exposed through inhalation to acrylonitrile concentrations of 50 and 240 mg/m^3 for 3 h a day, 6 days a week, for 6 months, showed significant defects in performance in a "Y" maze (Krysiak, 1971). Rats exposed to 10 mg/m^3, 5 days/week, 4 h a day, for 4 months, showed a 59% decrease in the activity of brain catalase, a 59% decrease in brain peroxidase, and a 37% decrease in -SH groups (Efremov, 1976d). Histopathological changes in the nervous system, consistent with a diagnosis of astrocytoma, were observed in rats exposed to acrylonitrile at 35, 100, or 300 mg/litre in drinking-water (Quast et al., 1977).

7.3.6 Kidney function

Knobloch et al. (1972) exposed rats to 50 or 240 mg/m^3, for 3 h per day, 6 days per week, for 6 months; kidney dysfunction was indicated by increased urinary output at both

concentrations and an increase in urinary protein and areas of degenerated proximal convoluted tubules at the higher concentration.

There were no abnormalities in the urine chemistry of rats drinking water containing acrylonitrile at 0, 35, 100, or 300 mg/litre (Quast et al., 1977), or of dogs (Quast et al., 1975), drinking water containing 100, 200, or 300 mg/litre. When acrylonitrile was applied to the skin of the tail of rats, daily, at doses of 2.82, 0.56, or 0.11 mg/kg body weight for 4.5 months, the excretion of urinary chlorides increased on day 10 of exposure at the 2 higher doses and decreased at the lowest dose (Zotova, 1976).

7.4 Teratogenicity and Embryotoxicity

The teratogenic potential of ingested or inhaled acrylonitrile was investigated by Murray et al. (1978). Groups of pregnant SD rats were given acrylonitrile at 0, 10, 25, or 65 mg/kg body weight per day, by gavage, from day 6 to day 15 of gestation. Groups of 30 pregnant SD rats were exposed 6 h per day to 0, 87, or 174 mg/m^3 (0, 40, or 80 ppm) acrylonitrile by inhalation, during the same period of pregnancy. A dose of 65 mg/kg body weight per day caused marked maternal toxicity, significant embryotoxicity, and an increased incidence of fetal malformations. Findings of the two studies suggesting a teratogenic effect were noted at 25 mg/kg per day and at 174 mg/m^3 (80 ppm). At 10 mg/kg body weight per day and 87 mg/m^3 (40 ppm), no embryotoxicity or teratogenicity was found. There was no apparent correlation between the degree of toxicity seen in the individual dams and the occurrance of malformations in their offspring.

Embryotoxic effects in pregnant mice of 3 strains were described after intraperitoneal administration of unspecified doses of acrylonitrile (Scheufler, 1976). A single ip injection of acrylonitrile of 32 mg/kg body weight, given on the 5th or 7th day of pregnancy, induced an embryotoxic effect in mice from an inbred strain of AB Jena-Hall, but not in DBA and C57 Cl mice (Scheufler, 1980).

Kankaanpää et al. (1979) studied the embryotoxic effects of acrylonitrile using chick eggs, but did not find any clear evidence of its teratogenicity.

The exposure of Sprague-Dawley rats to an acrylonitrile concentration in drinking-water of 500 mg/litre led to decreased fertility and decreased viability of the young, and the females developed a progressive muscular weakness in the hind legs about 16-19 weeks after the weaning of the second litter (Svirbely & Floyd, 1961).

Willhite (1981a,b) observed skeletal malformations in hamster fetuses after the administration of acrylonitrile at

80 mg/kg body weight to pregnant hamsters. The histological study of both early embryos and term fetuses revealed mesodermal changes, including a reduction in the number of cells, shrinkage of the cell cytoplasm, and enlarged extracellular spaces. In addition, a reduction in mitotic figures and focal necrosis were noted. The affected embryos were smaller and their development was delayed compared with untreated controls. Teratogenic effects were only observed when there was simultaneous maternal toxicity.

7.5 Mutagenicity

7.5.1 Bacterial systems

(a) Ames test with Salmonella typhimurium strains

Tests have been carried out on several strains, with and without metabolic activation, using several methods of treatment with acrylonitrile. Negative results were obtained, with and without activation, in 5 tested strains in 2 studies (Litton Bionetics, 1975; Stanford Research Institute, 1976). A weak, but reproducible, positive effect was observed with metabolic activation using between 0.5 and 1.5 mg acrylonitrile per plate in strain TA 1535 (Haskell Laboratory, 1975; De Meester et al., 1978, 1979). Three methods of exposure were examined by Milwy & Wolff (1977) in 3 strains of S. typhimurium. A low level of mutagenic activity was noted in strain TA 1535, when plates were sprayed with acrylonitrile or when it was mixed with the medium, with activation.

Exposure to acrylonitrile vapour of the strains TA 1535 and TA 100 also demonstrated acrylonitrile mutagenicity (Duverger-Van Bogaert et al., 1981; Ivanov, 1981). Zhurkov et al. (1983) tested the mutagenicity of acrylonitrile in strains TA 1535 and TA 1538, with and without microsomal activation, and found a dose-dependent effect in strain TA 1535.

Urine collected from rats and mice treated with acrylonitrile was mutagenic in S. typhimurium strain TA 1530, in the absence of metabolic activation. Pre-treatment of the animals with phenobarbital abolished the direct-mutagenicity of urine from rats and reduced that from mice. The addition of beta-glucuronidase (EC 3.2.1.31) to the incubation mixtures enhanced the mutagenic activity of urine from acrylonitrile-treated animals (Lambotte-Vandepaer et al., 1980, 1981a). Duverger-van Bogaert et al. (1982b) suggested that glutathione might play a role in the formation of a mutagenic metabolite of acrylonitrile. The mutagenic activity of acrylonitrile vapours towards S. typhimurium strains was strictly dependent on the presence of an activation system, confirming the report of Milwy & Wolff (1977). Lambotte-Vandepaer et al. (1980)

indicated that animal urine might retain its mutagenic activity for as long as a week after collection. The acrylonitrile-derived epoxide, glycidonitrile, synthesized by Kopecký & Smejkal (unpublished data, 1979), was shown to be the principal substance that exerted mutagenic activity in the absence of metabolic activation, whereas acrylonitrile itself required metabolic activation in the S. typhimurium Ames test (Cherna et al., 1981).

(b) Mutagenicity in Escherichia coli

One of 3 strains of E. coli (WP2) revealed mutagenic activity of acrylonitrile; activation did not have any effect (Venitt et al., 1977). The mutagenic activity of acrylonitrile was confirmed in other experiments using the simplified fluctuation test of Green et al. (1976). The results suggested that acrylonitrile caused non-excisable mis-repair of DNA associated with the generation of DNA strand breaks (Venitt et al., 1977). The method of Slater et al. (1971) did not reveal any effect with or without an activation system at 10 µg acrylonitrile per plate (Litton Bionetics, 1976).

The variability of the results, even when the same kinds of assays are used, could be because of differences in purity of the acrylonitrile, in method, or in bacterial sensitivity. However, the mutagenicity of acrylonitrile in bacterial systems seems to have been established.

7.5.2 Yeast assays

Possible mutagenic activity of acrylonitrile was found with Saccharomyces cerevisiae, but metabolic activation was without effect (Litton Bionetics, 1975).

7.5.3 Drosophila melanogaster

A negative result was obtained when 0.1% acrylonitrile was administered by intra-abdominal injection into D. melanogaster in order to examine its ability to induce a recessive lethal effect in the X chromosomes (Benesh & Shram, 1969).

7.5.4 Mammalian cell in vitro assays

The L5178Y kinase mouse lymphoma cell assay (Litton Bionetics, 1976) failed to show mutagenic activity of acrylonitrile using the procedure of Clive & Spector (1975). Chinese hamster ovary cells showed an increase in sister chromatid exchange (SCE) after exposure to acrylonitrile, when

co-cultured with rat hepatocytes (Ved Brat & Williams, 1982). No effect was found without the latter.

Acrylonitrile induced a slight increase in the SCE of cultured human lymphocytes in the presence of S-9 mix and increased unscheduled DNA synthesis with a very high concentration (0.5 M) (Perocco et al., 1982). Application of acrylonitrile to primary Syrian golden hamster embryo cells in culture produced foci of morphologically-transformed cells. Pre-treatment with simian adenovirus (SA7) caused an 8 to 9-fold increase in the frequency of virus-transformed foci. When ^{3}H-thymidine-labelled cells were treated with acrylonitrile and their DNA subjected to alkaline sucrose gradients, a shift in the sedimentation pattern occurred, which was reminiscent of that observed with carcinogen treatment. These observations added support to recent studies indicating that acrylonitrile may be carcinogenic (Parent & Castro, 1979).

7.5.5 Mammalian in vivo assays

The inhalation exposure of 16 Sprague-Dawley male rats to acrylonitrile levels up to 1085 mg/m^3 (500 ppm) for 90 days did not reveal chromatid or chromosomal aberrations or bone-marrow abnormalities (Johnson et al., 1978). The results of Rabello-Gray & Ahmed (1980) and the recent results of Leonard et al. (1981) also showed that acrylonitrile fails to induce chromosomal aberrations in somatic and germ cells.

Similar negative results were reported by Zhurkov et al. (1983) following inhalation exposure of mice to acrylonitrile concentrations of both 100 mg/m^3 and 20 mg/m^3. They also reported negative results in a dominant lethal assay in mice.

From preliminary results concerning DNA-alkylation by acrylonitrile and vinyl chloride monomer (Peter et al., 1983), it appears that DNA-alkylation occurs to a much lesser extent with acrylonitrile than with vinyl chloride monomer. This is consistent with the absence of mutagenic effects in vivo.

7.6 Carcinogenicity

Although full data were not available to the Group, there was strong evidence from the data considered that acrylonitrile is a carcinogen in rats.

Maltoni et al. (1977, 1982) investigated the carcinogenicity of acrylonitrile administered to Sprague-Dawley rats by inhalation at 87, 44, 22, and 11 mg/m^3 (40, 20, 10, and 5 ppm), 4 h daily, 5 times a week, or by stomach tube as a solution in olive oil, at a dose of 5 mg/kg body weight, once a day, 3 times a week. In each case, the rats were treated for 52 weeks and then kept without further

treatment until death. An increased incidence of some tumours was noted in the acrylonitrile-treated animals, e.g., mammary tumours, forestomach papillomas and acanthomas, and encephalic tumours (gliomas).

Two-year studies on Sprague-Dawley rats, following inhalation exposure to acrylonitrile or ingestion in drinking-water, have been performed at the Dow Chemical Company laboratories (Quast et al., 1980a,b). In the inhalation studies, rats were exposed to 0, 44, or 174 mg/m^3 (0, 20, or 80 ppm) for 6 h/day, 5 days a week, for 24 months. Treatment-related tumours were found in the central nervous system, Zymbal gland, tongue, stomach, small intestine, mammary gland, and nasal turbinates. An apparent decrease in tumours of the pituitary gland, the adrenals, the thyroid, the pancreas, and testes was observed in the exposed rats.

In the ingestion study, rats were maintained on water containing acrylonitrile levels of 0, 35, 100, and 300 mg/litre, equivalent to mean dosage levels of 0, 4, 9, or 22 mg/kg body weight per day. Evidence of oncogenicity was found in rats at all dose levels of acrylonitrile. An increased tumour incidence was observed in the treated rats affecting, particularly, the central nervous system and also the Zymbal gland, tongue, stomach, small intestine, and mammary gland. A decreased incidence of tumours was observed at some sites: pituitary, thyroid, adrenals, pancreas, and uterus.

In studies performed by Hogen & Rinehart (1980), acrylonitrile was administered to Sprague-Dawley rats in the drinking-water at 1 or 100 mg/litre for 19-22 months, or by gavage at 0.1 or 10 mg/kg body weight per day in water for about 20 months. A second group of Fisher 344 rats received acrylonitrile in the drinking-water at 1, 3, 10, 30, or 100 mg/litre for 23-26 months. A statistically significant increase in tumours was reported in the group receiving acrylonitrile at 10 mg/kg body weight by gavage and in the groups receiving 10, 30, or 100 mg acrylonitrile/litre drinking-water.

So far, no information is available on the carcinogenicity of acrylonitrile for animal species other than rats.

After reviewing these data, IARC (IARC, 1982) and COC (UK Ministry of Agriculture, Fisheries and Food, 1982) concluded that acrylonitrile was a carcinogen in experimental animals.

8. EFFECTS ON MAN

Acrylonitrile has long been known to be a toxic substance that induces systemic as well as local injury in both animals and man. It has frequently been used in combination with other chemicals; they may modify its toxicity, as was the case when it was used as a fumigant.

8.1 Acrylonitrile

8.1.1 Acute Toxicity

8.1.1.1 Inhalation exposure

A 22-year-old chemist, who was exposed to acrylonitrile vapours when a distillation apparatus leaked, developed headache, vertigo, vomiting, tremors, uncoordinated movements, and convulsions (Sartorelli, 1966). Vomiting and nausea persisted for 24 h. One day after exposure, slight liver enlargement and congestion of the oral pharynx, but no disorders of the CNS, were noted. After 4 days, no kidney, liver, cardiac, or respiratory abnormalities were detected. Workers exposed to "mild" concentrations of acrylonitrile in synthetic rubber manufacture developed nausea, vomiting, weakness, nasal irritation, and an "oppressive feeling" in the upper respiratory tract (Wilson, 1944). Headache, fatigue, and diarrhoea were observed in some cases, and mild jaundice lasting for several days and accompanied by liver tenderness and low-grade anaemia in a few others. Jaundice lasted for 4 weeks in 1 case; this individual complained of lassitude and fatigue after one year. Zeller et al. (1969) observed that in 16 cases of acute inhalation of acrylonitrile fumes by workers, nausea, vomiting, headache, and vertigo appeared within 5-15 min; none of the workers needed hospitalization. The authors described 50 cases of skin contact with irritation, erythema, and blistering appearing within 5 min to 24 h, but with no systemic consequences. Workmen exposed to concentrations varying from 35 to 220 mg/m^3 (16-100 ppm) for 20-45 min during cleaning operations in polymerizers frequently complained of a dull headache, fullness in the chest, irritation of the eyes, nose, and throat, and feelings of apprehension and nervous irritability. Some workmen had "intolerable itching" of the skin, but no accompanying dermatitis.

8.1.1.2 Dermal exposure

A male laboratory worker who spilled "small quantities" of liquid acrylonitrile on his hands, developed diffuse erythema on both hands and wrists after 24 h, and blisters on the fingertips by the third day. The hands were slightly swollen, erythematous, itchy, and painful. The fingers remained dry and scaly on the 10th day (Dudley & Neal, 1942). Wilson et al. (1948) observed that direct skin contact led to irritation and erythema followed by scab formation; healing was slow. Development of allergic dermatitis is possible; a 27-year-old individual developed a rash on his finger following the use for 6 weeks of a finger splint made from an acrylonitrile/methyl methacrylate copolymer. Patch testing gave positive reactions to the copolymer and 0.1% acrylonitrile (Balda, 1975). In another case report, skin lesions were first observed at the site of contact with liquid acrylonitrile, which then spread rapidly to other neighbouring regions. Several days after contact, the lesions spread rapidly to other parts of the body that had not been exposed, and these extensions were assumed to be an allergic reaction (Hashimoto & Kobayashi, 1961).

In addition to local dermal toxicity, dermal absorption of acrylonitrile may lead to systemic poisoning. Grunske (1949) described a fatal case in which a 3-year-old girl had entered a room that had recently been sprayed with an acrylonitrile-containing insecticide (Ventox). Exposure was mainly through inhalation, but skin exposure was possible, too. Another fatal case was reported by Lorz (1950) in which a 10-year-old girl had been treated on the scalp for lice with an insecticide that was identified as containing acrylonitrile (Ventox). She had impetigo and widespread scratches on the skin of the scalp. This could have increased the absorption of acrylonitrile.

Two workers who spilled liquid acrylonitrile on their legs, immediately washed their legs and dried their shoes, but put them on again. Blisters developed at the sites of contact, 6-8 h after the spill. Therapy lasted 21 and 38 days, respectively. The skin of 2 workers who were cleaning apparatus (temperature 50 °C), came into contact with 5% acrylonitrile solution; other possible substances in the mixture were not specified. Serious skin burns developed. Therapy lasted 35 and 72 days, respectively (Babanov, 1957). Zeller et al. (1969) reported 50 cases of skin damage resulting from occupational contact with acrylonitrile. A burning sensation developed within 5 min to 24 h followed by a reddening of the area, which often blistered after 1 day.

8.1.2 Chronic toxicity - occupational exposure

Chronic effects can potentially occur after prolonged exposure to acrylonitrile, both in the vapour and liquid forms.

8.1.2.1 Clinical observations

Complaints of poor health, headache, decreased work capacity, poor sleep, irritability, chest pains, poor appetite, and skin irritation (during the first months of employment only) came from workers employed in the manufacture of acrylonitrile (Zotova, 1975a).

In a study by Sakurai & Kusumoto (1972), workers employed in acrylonitrile manufacture also complained of headache, weakness, fatigue, nausea, vomiting, nosebleeds, and insomnia; the symptoms correlated well with the length, but not with the level of exposure or with the age of the workers. A total of 4439 examinations were made over about 10 years prior to 1970, in 576 workers who formed 2 cohorts, one exposed to concentrations of acrylonitrile below 11 mg/m^3 (5 ppm), the other below 45 mg/m^3 (20 ppm). However, the authors later stated that these exposure levels were not reliably reported (Sakurai et al., 1978).

Babanov et al. (1959) reported that workers exposed to acrylonitrile concentrations at 0.6-6 mg/m^3 for approximately 3 years suffered from headache, insomnia, pains in the heart region, general weakness, decreased working capacity, and increased irritability. The vocal cords were inflamed, and non-specific changes in the vestibular apparatus and pale mucous membranes and skin were seen. Blood pressure was said to be reduced.

Changes in the health status and laboratory tests were not observed in a group of 23 men who had been working for 3-5 years in an acrylonitrile plant, where, during the warm season, exposure levels reached 4.2-7.2 mg/m^3 (Gincheva et al., 1977). Stamova et al. (1976) studied workers' health in the related polyacrylic fibre plant in which acrylonitrile exposure levels ranged around 10 mg/m^3, but could fluctuate upwards to 25 mg/m^3. Workers were also exposed to other chemical substances. An increase was found in both skin diseases and various "neurasthenic" complaints and diseases. Dorodnova (1976) did not find any differences in the gynaecological health status of 410 women working in a polyacrylic fibre plant in Saratov compared with that of 436 unexposed women.

8.1.2.2 Haematology

Compared with the findings in blood donors, some male and female employees exposed to acrylonitrile at 2.5-5 mg/m^3 showed a reduced haemoglobin level, erythrocyte count, leukocyte count, and percentage of neutrophiles, with an increased percentage of lymphocytes and plasma iron. Inhibition of maturation of normoblasts in bone marrow was also reported (Shustov, 1968). Similar results were reported by Zotova (1975b). Lower erythrocyte, haemoglobin, and total white counts were found in laboratory workers exposed to acrylonitrile, and in apparatus operators and machinists. Higher than normal total glutathione levels were found in male operators and maintenance men and reduced glutathione levels in male apparatus operators. Oxidized glutathione was elevated and total sulfhydryl groups decreased in workers employed in all these occupations.

Lower erythrocyte counts and a relative lymphocytosis were also observed by Babanov et al. (1959) in the study mentioned above.

8.1.2.3 Other organs

(a) Liver

Sakurai & Kusumoto (1972) (section 8.1.2.1) reported some abnormal results in liver function tests; however, in a further study of 102 workers from some of the factories, Sakurai et al. (1978) did not find any significant liver function test abnormalities related to acrylonitrile exposure, when exposure levels had decreased from 11-44 mg/m^3 (5-20 ppm) to 9 mg/m^3 (4.2 ppm). Increased serum cholinesterase activity, hyperbilirubinaemia, decreased coloidal stability, and hypergammaglobulinaemia were described in workers exposed to acrylonitrile concentrations of up to 5 mg/m^3 and to acrylonitrile-polymer dust of up to 1.5 mg/m^3 (Enikeeva et al., 1976). These effects have not been reported elsewhere.

(b) Eye

Blepharoconjunctivitis was reported by Delivanova et al. (1978) in all of 302 workers examined over a 2-year period; 42 had severe alterations caused by conjunctivitis, and all disorders were connected with exposure to acrylonitrile.

(c) Gastro-intestinal effects

Symptoms of gastritis and colitis were observed in workers exposed to acrylonitrile concentrations of up to 5 mg/m^3 (Enikeeva et al., 1976).

(d) Immune system

Acrylonitrile has been found to have an immunodepressive effect. The functional activity of T-lymphocytes was found to have decreased in workers exposed to acrylonitrile (Ivanov, private communication, 1983).

8.1.2.4 Nervous system

Nausea, vomiting, headache, and vertigo (Wilson, 1944; Wilson et al., 1948; Zeller et al., 1969; Sakurai & Kusumoto, 1972; Zotova, 1975) indicate a possible effect of acrylonitrile on the nervous system. Ageeva (1970) reported a significant decrease in an "epinephrine-like substance", and an increase in acetylcholine. Depression, lability of autonomic functions (lowered arterial pressure, labile pulse, diffuse dermographia, increased sweating, change in orthostatic reflex) were also observed in workers involved in acrylonitrile production.

8.1.2.5 Dermal effects

Spassovski (1976) reported irritant and allergic dermatitis in acrylonitrile workers; dermatitis was also observed by Antonev & Rogailin (1970) and Stamova et al. (1976).

8.2 Mutagenicity

Thiess & Fleig (1978) examined workers who had been exposed to acrylonitrile for 15.3 years and workers who had not been exposed. No differences were found in the incidence of chromosomal aberrations, including or excluding gaps, in the 100 metaphases examined for each person.

8.3 Carcinogenicity

In a retrospective cohort epidemiological study of 1345 male workers with potential exposure to acrylonitrile from 1950-66, followed until 31 December, 1976, 25 cases of cancer were found with 20.5 expected, based on company rates (O'Berg, 1980). Of these, 8 were respiratory cancer cases, with 4.4 expected. Twenty-three cases occurred among workers first

exposed during the start-up period (1950-52) when exposures were higher; only 12.9 were expected (P = 0.01).

The standardized incidence ratio (SIR) was 179 for cancer among the operators and mechanics who had at least 6 months' exposure and began their assignments during start-up. A "dose response" was shown with those with longer duration of employment, workers with estimated higher exposures having higher risk. Latency was also demonstrated, with 17 of the 24 cases occurring 20 years after the onset of exposure among those with at least 6 months' employment, including 6 of the 8 lung cancer cases. It should be pointed out that, using the National Cancer Institute's expected incidence rates for 1969-71, the expected rate would be 25.5 rather than 20.5 from the company rates. In a concomitant cancer mortality study, 20 cancer deaths were found with 17.4 expected, using company rates (not significant); the expected rates exceeded the company rates using national, state, or regional cancer rates. The author felt that it might be premature to evaluate mortality statistics, because of insufficient latency (many cancer cases had been recently diagnosed and were still living). Smoking habits were not considered, though the author stated that 7 out of the 8 lung cancer cases were known to have smoked.

A follow-up study on the mortality rate among 327 employees of a chemical rubber plant in the USA revealed that the number of deaths from lung cancer was significantly higher than expected (9 versus 5.9 for US white males and 4.7 for other rubber workers from the same city). The greatest excess was seen among men who had worked for 5-14 years and who had started working there at least 15 years before death (Delzell & Monson, 1982). This study was confounded by the multiple exposure of workers in the nitrile rubber manufacturing plant.

Kiesselbach et al. (1979) examined the mortality rate, the cancer rate, and the type of cancer against the period of exposure to acrylonitrile in 884 workers. The results revealed that the general mortality of the exposed group was markedly lower than that of the normal population (58 versus an expected 104), possibly because of the "healthy worker" effect. The mortality rate for malignant tumours, cardiovascular, brain, respiratory, and gastro-intestinal diseases, suicide, and other causes was the same as in the normal population. No relationship was found between length of exposure and mortality from tumours.

An excess of deaths from lung cancer was reported in acrylonitrile workers by Thiess et al. (1980). In addition, 2 cases of Hodgkin's disease contributed to a slight excess of cancer of the lymphatic tissue. However, exposure to other substances, some of them known carcinogens, made interpretation of the results difficult.

A cohort study on men potentially exposed to acrylonitrile during the start-up of a plant indicated that there was no excess mortality from lung cancer. There were no deaths from lung cancer in maintenance workers, who possibly had the highest exposures. There was an excess of kidney cancers (based on 2 cases only) and of circulatory disease other than rheumatic and atherosclerotic (based on 5 cases), accompanied by a deficit of atherosclerotic heart disease.

Because the cohort was small with only 4 cancer deaths observed, it could not give an indication of excess cancer risk or association with duration of exposure to acrylonitrile. An additional retrospective cohort mortality study, in two acrylonitrile plants in the USA, on 352 males exposed for 6 months or more prior to 1968 and followed up until December 1977, did not show any excess mortality including cancer mortality. There were 15 deaths from all causes, 18.11 being expected, and 3 deaths from cancer (2.8 expected) (Zack, unpublished data, 1980).[a]

In a study by Nakamura (1981), 9525 workers employed in the production of acrylonitrile, acrylonitrile rubbers, and ABS were studied. Deaths due to cancers in general and to lung and colon cancers in particular, were not increased, while 7 deaths due to liver, gall bladder, or cystic duct cancer were found against the 5 that might have been expected.

The mortality of 1111 men who worked on the polymerization of acrylonitrile and the spinning of acrylic fibre in the United Kingdom from 1950 to 1968 was surveyed up to the end of 1978. Seventy-nine deaths were identified in 6 factories. The total number of deaths among men exposed to acrylonitrile for at least one year was slightly lower than expected (68 versus 72.4) and a relative excess of deaths from all cancers was found, arising mainly from cancers of the lung, stomach, colon and brain, pancreas, testis, and bladder (21 versus 13 expected). The authors considered particularly relevant, the excess of lung cancer in those aged 15-44 years. Nevertheless, the authors considered that their results were inconclusive and urged continued surveillance and analysis of the exposed population in the United Kingdom (Werner & Carter, 1981).

The epidemiological studies provide some indications that acrylonitrile exposure is associated with cancer, particularly of the lung. However, the studies reported, while neither conclusive nor contradictory, are limited by insufficient latency. Other difficulties, such as cohort identification

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[a] Zack, J.A. (1980) The mortality experience of Monsanto workers exposed to acrylonitrile (Monsanto Internal Report).

and selection, and combined exposures have made interpretation difficult. Further epidemiological data are therefore of great importance, and consideration of smoking and single exposures to acrylonitrile is desirable.

8.4 Simultaneous Exposure to Acrylonitrile and Other Chemicals

8.4.1 Acute toxicity

Numerous non-fatal and fatal cases of poisoning by acrylonitrile-containing mixtures have been described (Davis et al., 1973). In home fumigation, an acrylonitrile mixture with carbon tetrachloride or methylene chloride is placed in shallow open pans and the vapours dispersed by fans for 24-72 h, the operator deciding when the house is safe for occupancy (Davis et al., 1973). A man working in the polymerization of acrylonitrile, polybutadiene, and styrene for 2 years complained of numbness of the fingers and toes, severe fatigue in the lower extremities, and general malaise. Decreased patellar and achilles tendon reflexes, and hypoaesthesia in the peripheral parts of the fingers and toes were observed. Free acrylonitrile was detected in the urine. The exposure level of acrylonitrile was estimated to exceed 108.5 mg/m^3 (50 ppm) (Seki, 1967).

Lachrymation, burning in the throat, coughing, sneezing, nausea, vomiting, dizziness, visual disturbance, headache, coma, seizures, and dermatitis have been described in non-fatal cases (Davis et al., 1973). Fatalities have occurred following exposure to vapours (Grunske, 1949; Davis et al., 1973) and liquid (Lorz, 1950). Cyanide was detected in the blood in some cases. Symptoms and signs prior to death varied from case to case; sore throat, weakness, dizziness, vomiting, eye irritation, respiratory disorders, pallor, tachycardia, tremors, unconsciousness, and epidermal necrolysis have been described. Other pathological conditions occurred, but many could have been the result of pre-existing disease or of exposure to the other component(s) of the mixture. Findings in children suggest that they may be more sensitive to acrylonitrile exposure than adults (Grunske, 1949).

8.4.2 Chronic toxicity

Abnormalities in subjects exposed simultaneously to acrylonitrile and several other chemicals have been described in several studies. As the concentrations of the other chemicals were frequently higher than those of acrylonitrile, it is difficult to decide whether the abnormalities were

caused by acrylonitrile, the other chemical(s), or a combination of the two.

An abnormally high proportion of workers exposed to acrylonitrile levels of 3-20 mg/m^3, 33 ppm NH_3, up to 1 mg H_2SO_4/m^3, 0.41-0.67 mg NaOH/m^3, and 2-10 mg acetic acid/m^3 were described as suffering from a variety of symptoms ascribed to disorders of the autonomic nervous system ("neurasthenic syndrome") (e.g., irritability, headache, poor appetite, fatigue). Intolerance to alcohol has also been observed (Orusev & Popovski, 1973; Orusev et al., 1973).

Apprentices exposed to acrylonitrile (0.8-1.8 mg/m^3), methyl methacrylate (16-17 mg/m^3), and sodium thiosulfate were examined before exposure, after 1-2 weeks, and after a further unspecified time. "Neurasthenic" symptoms were rare before, but frequent after exposure, and the incidence of immunological reactivity against the chemicals increased, as did the concentration of ceruloplasmin (Mavrina & Il'ina, 1974). Dermal tests for allergy were also made by Hromov (1974) in workers who had been in contact with acrylonitrile, methyl acrylate, and sodium thiocyanate. Intradermal samples showed positive haemoagglutination reactions in 86.5% of workers exposed to acrylonitrile, 76.1% exposed to methyl acrylate, and 53.6% exposed to sodium thiocyanate. Dermatitis, eczema, and urticaria occurred.

Mavrina & Hromov (1974) and Shustov & Mavrina (1975) reported abnormalities of the liver, nervous, cardiovascular, and gastrointestinal systems in workers occupationally exposed to acrylonitrile, methyl acrylate, and sodium thiocyanate during fibre production. In particular, symptoms associated with the activity of the autonomic nervous system were noted among the 340 workers examined. Dryness, desquamation, fissures, and diffuse erythema of the skin were also apparent. Women exposed to acrylonitrile and methyl acrylate (Chobot, 1979) were said to suffer from disturbances in menstrual function twice as frequently as a control group. A low incidence of irritant and allergic dermatitis and vitiligo was noted in workers exposed to acrylonitrile, methyl acrylate, and dimethylformamide in fibre production (Bainova, 1975). In 11 out of 28 workers, delayed skin sensitization and allergic dermatitis were observed with dimethylformamide.

Ostrovskaja et al. (1976) observed workers exposed to acrylonitrile, acetonitrile, and hydrocyanic acid during training and after 1.5 years and 3 years. In 190 men and women, aged 20 - 30 years, many signs and symptoms were noted including modified reflexes, changes in blood pressure, ECG, and EEG. However, it is difficult to attribute the findings of these authors solely to acrylonitrile exposure.

9. EVALUATION OF HEALTH RISKS TO MAN FROM EXPOSURE TO ACRYLONITRILE

9.1 Sources and Levels of Exposure

Acrylonitrile is a colourless, volatile, chemically reactive liquid; it does not occur as a natural product. The monomer is used world-wide, on a large scale, in the manufacture of polymers, fibres, and rubbers and as a chemical intermediate. Acrylonitrile-containing polymers have been used in the manufacture of products that come into contact with food; the amounts of acrylonitrile that migrate into foods can be reduced to negligible quantities by the use of good manufacturing practices in the production of the polymers.

The major sources of contamination of the general environment are acrylonitrile-producing and -polymerizing plants. The occurrence of acrylonitrile in air, water, and soil near industrial plants has been described. There is evidence that acrylonitrile has persisted in soil for long periods following accidental spillage; subsequent contamination of ground water has been demonstrated.

The highest exposures occur in the workplace. Experience shows that containment of such exposures can more readily be achieved in production plants than in those in which acrylonitrile is used to make other products. In a number of countries, exposure limits or recommended limit values for the workplace have been arrived at; values recently set have tended to be lower than in the past (Table 12).

Accidental exposure to acrylonitrile liquid and vapour may occur during the various stages of production, transport, and use.

9.2 Acrylonitrile Toxicity

Inhaled acrylonitrile vapour is readily absorbed. Acute systemic effects following absorption of vapour have been described. Symptoms were non-specific and referable to the gastrointestinal and respiratory tracts, the liver, and the central nervous system. No acute adverse effects have been reported following daily exposure (8-h) to up to 45 mg/m^3.[a] At higher concentrations rising to 220 mg/m^3, 20-40 min exposure resulted in complaints of headache,

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[a] This value was for many years the occupational exposure limit in many countries where acrylonitrile was manufactured.

Table 12. Occupational exposure limits for selected countries

Country	TWA mg/m^3	STEL mg/m^3	Reference
France	9	34***	INRS (1983)
Germany, Federal Republic of	-* (S)		DFG (1982)
Hungary	0.5	0.5	Hungary (1979)
Japan	45	-	Japan Association of Industrial Health (1972)
Poland			Poland (1982)
Sweden	4** (S)	13	Sweden (1981)
United Kingdom			
USA	4.5** (S)		ACGIH (1982)
USSR		0.5 (S)	USSR (1982)

* Proved animal carcinogen, strongly suspected of also being carcinogenic for human beings. No safe concentration can be listed.

TWA = Time-weighted average.
STEL = Short-term exposure limit.
** Listed as Industrial Substance Suspect of Carcinogenic Potential for Man.
*** Alarm level.
S = Skin uptake can contribute to overall exposure.

irritation of the upper respiratory tract and the eyes, nervous irritability, and itching of the skin. Fatalities have been reported following the use of fumigant mixtures containing acrylonitrile together with carbon tetrachloride and methylene chloride. Exact exposure conditions are not known, but animal data suggest that inhalation exposure to acrylonitrile at 500-2000 mg/m^3 for 1/2-3 h could be fatal. Simultaneous exposure to some organic solvents may enhance the toxicity of acrylonitrile.

Liquid acrylonitrile is also absorbed through the skin, reportedly giving rise to non-specific symptoms similar to those that follow acrylonitrile vapour inhalation. Local injury can occur a few hours after exposure to liquid acrylonitrile. One fatality has been reported. It is also an irritant to the eye.

Skin absorption of vapour does not appear to contribute significantly to overall acrylonitrile uptake in the workplace.

There are no indications that acrylonitrile accumulates in the body following prolonged exposure to levels found in the workplace.

Skin sensitization has been reported in a few cases; however, no evidence is available to suggest the occurrence of pulmonary allergic reactions.

Acrylonitrile has been shown to induce embryotoxic and teratogenic effects at high dosage levels in experimental animals.

Although acrylonitrile is metabolized partly to cyanide, it has been demonstrated that the acute toxic actions of acrylonitrile are not solely due to cyanide, as was once believed.

Acrylonitrile has been shown to be mutagenic in some *in vitro* systems in the presence of metabolic activation systems. So far, mutagenic activity has not been demonstrated in *in vivo* assay systems.

Complaints of ill health in workers exposed for a number of years to acrylonitrile concentrations of less than 45 mg/m^3 have been reported in several studies. The complaints were variable in nature and no consistent correlation with the extent of exposure appears to have been established. The studies do not provide evidence of a specific disease arising from long-term, low-level exposure.

Several long-term studies in which acrylonitrile was administered to rats orally and by inhalation demonstrated the induction of malignant tumours. Data available in summary form suggest that the incidence was dose-related. Eight epidemiological studies have been carried out on workers exposed to acrylonitrile. These studies have not demonstrated conclusively that there is a correlation between exposure to acrylonitrile and the incidence of cancer in man. Nevertheless, the findings are not incompatible with the supposition that acrylonitrile is a potential human carcinogen and thus give no cause for disregarding the evidence that has been provided by animal studies.

It is not possible to establish a level below which no adverse effects occur on the basis of the experimental and epidemiological data presented in this document. However, it is evident that exposure to acrylonitrile should be kept as low as possible in both the workplace and the general environment, and that skin contact with the liquid should be avoided.

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