Evaluation of the Genotoxicity of Acrylonitrile in Different Tissues of Male Mice

Maha A. Fahmy

Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

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Summary Acrylonitrile (ACN) is an extensively produced industrial chemical used in the synthesis of plastics and fibers. It has been found in car exhaust, cigarette smoke, drinking water and food products. The ability of acrylonitrile to induce sister chromatid exchanges (SCE's) and chromosomal aberrations in male mice was investigated. Three dose levels of acrylonitrile (5, 7.5, 10 mg kg⁻¹ b.wt.) were tested (i.p.) for SCE's. The dose 10 mg kg⁻¹ b.wt. induced significant increase in the frequency of SCE's 7.2 ± 0.2 /cell as compared with 4.4 ± 0.26 for control, but the rate of increase was significantly lower than that with mitomycin C 11.9 ± 0.5 . Oral treatment with acrylonitrile induced a statistically significant increase in the percentage of chromosomal aberrations in mouse spermatocytes, bone-marrow as well as spleen cells. The aberration frequency increased with the dose and the number of treatments. In all experiments, mitomycin C induced much higher effect.

In conclusion acrylonitrile has shown clastogenic potential in the mice examined.

With the rapid industrial development, thousands of chemicals are being used. Many of them are toxic which can pollute the environment (air, land, water) and many human bodies through occupational exposure.

Acrylonitrile (ACN) is an extensively produced industrial chemical used in the manufacture of acrylic fiber, nitrile rubber, resins and plastics. It is used as an intermediate in the synthesis of antioxidants, surface coatings, adhesives and dyes (Duverger–Van Bogaert *et al.* 1981). Moreover ACN had been used as a fumigant pesticide for stored grains and fruits (IARC 1979). In clinical practice acrylonitrile is used in the manufacture of high permeable dialysis tubing (Ward *et al.* 1993) and for synthesis of an artificial membrane to encapsulate Langerhans islets implants (Kessler *et al.* 1992). ACN is an environmental contaminant, which has been found in contaminated drinking water (Rubio *et al.* 1990) as well as food products infiltrated by ACN monomers from packing materials (Conacher *et al.* 1993). Cigarette smoke, car exhaust and industrial dust in work places have shown to contain many aliphatic nitriles including ACN (Baker *et al.* 1984, Byrd *et al.* 1990, Kondakova *et al.* 1990, Solionova *et al.* 1992). A significant increase in stomach and prostate cancer was observed in workers exposed to acrylonitrile (Werner and Carter 1981, Chen *et al.* 1988). Acrylonitrile was reported to be mutagenic in *Salmonella typhimurium* in the presence of liver S-9 fraction (Milvy and Wolff 1977) and in Chinese hamster ovary (Lambotte–Vandepaer and Duverger–Van Bogaert 1984). Recently ACN was reported to be positive in the yeast of *Saccharomyces cerevisiae* deletion (DEL) assay (Carls and Schiestl 1994).

In the present investigation, an attempt has been made to show the effect of acrylonitrile on mammalian chromosomes using the two cytogenetic parameters, sister chromatid exchange in bone-marrow and chromosomal aberration analysis in both somatic (bone-marrow and spleen) and germinal cells (spermatocytes) of male mice.

Materials and methods

Animals

Mature male Swiss mice (9–12 weeks old, weighing 25–30 g) were used in all experiments. The animals were obtained from a closed random bred colony at the National Research Centre, maintained under controlled conditions of temperature and humidity and received feed and water *ad libitum*.

Chemicals

Acrylonitrile ($\text{CH}_2=\text{CH}-\text{CN}$, Vinyl Cyanide) BDH Chemicals Poole [England]; M. weight 53.06 Stabilized with 0.005% methoxyphenol. Impurity 99% minimum assay.

Dosage and treatment

Sister chromatid exchange The experimental procedure was conducted in accordance with the protocol of Allen (1982). The used doses were 5, 7.5 and 10 mg kg^{-1} b.wt. acrylonitrile. BrdU tablets, weighing approximately 55 ± 2 mg, were placed subcutaneously. Mice were injected (i.p.) with acrylonitrile 8 hr following BrdU treatment and with colchicine 2 hr prior to killing. Metaphase spreads were prepared using the method of Yosida and Amano (1965). Differential staining of sister chromatids was performed according to the fluorescence plus Giemsa method of Goto *et al.* (1978) with the use of black lights. Only euploid cells ($2n=40$) and well spread chromosomes were selected for scoring. 40 metaphase spreads per animal were examined microscopically for SCE's (200 metaphases per each treatment group). In all experiments, acrylonitrile was dissolved in distilled water.

Chromosomal aberration For spermatocytes, bone-marrow or spleen, single oral treatment *via* oral gavage was administered. Three different dose levels, 7.75, 15.5 and 31 mg kg^{-1} b.wt. (1/8, 1/4, 1/2 LD_{50}) were used and mice killed 24 hr after treatment. Oral LD_{50} was determined experimentally. The dose 7.75 mg kg^{-1} b.wt. acrylonitrile was given daily for 5 consecutive days and cell samples from spermatocytes, bone-marrow and spleen were taken 24 hr after the third treatment and 24 hr or 7 days after the fifth treatment. Untreated mice and mice injected with mitomycin C 1 mg kg^{-1} b.wt. were used as controls. Control and treated animals were injected with colchicine 2–3 hr before killing. Primary spermatocytes at metaphase I (MI) were prepared from testes according to the air drying technique of Evans *et al.* (1964). Bone-marrow metaphases were prepared according to Yosida and Amano (1965). For spleen cells, fresh spleen was washed with RPMI medium and cut into small pieces. The cells were aspirated into a centrifuge tube and 0.075 M KCl was added. The cells were centrifuged at 1000 rpm for 10 min and fixed in methanol–acetic acid (3 : 1) (Amer *et al.* 1993). Slides were stained with 7% Giemsa in phosphate buffer. Around 75 metaphases were analysed per animal in five mice per group scoring the different types of chromosomal aberrations. Significant differences from controls were calculated with Student's t-test.

Results and discussion

The ability of acrylonitrile to induce sister chromatid exchanges (SCE's) in mouse bone-marrow cells was investigated (Table 1). SCE's are currently recognized as being a sensitive indicator of agents which damage mammalian DNA (Latt 1974) and their formation has been compared with that of mutation (Carrano *et al.* 1978, Wilkoskey and Rynard 1990). Three different dose levels of acrylonitrile (5, 7.5, 10 mg kg^{-1} b.wt.) were tested. The lowest dose tested had no significant effect with respect to SCE's while the higher doses increased the frequency of SCE's significantly. The mean frequency of SCE's reached 7.5 ± 0.21 per cell after treatment with the highest dose which is less than twofold increase as compared with the negative control 4.4 ± 0.26 . Comparing such fre-

Table 1. Frequency of sister chromatid exchanges (SCE's) in mouse bone-marrow cells induced after i.p. treatment with different concentrations of acrylonitrile

Treatment and doses	Mice No.	Metaphases with SCE's No.	SCE's No.	SCE's/Cell Mean $\pm$ S.E.
I. Control (non treated)	5	200	880	4.4 $\pm$ 0.26
II. Acrylonitrile 5 mg kg ⁻¹ b.wt.	5	200	1068	5.34 $\pm$ 0.17
7.5 mg kg ⁻¹ b.wt.	5	200	1244	6.22 $\pm$ 0.35*
10 mg kg ⁻¹ b.wt.	5	200	1499	7.50 $\pm$ 0.21**
Mitomycin C 1 mg kg ⁻¹ b.wt. (+ve Control)	5	200	2381	11.9 $\pm$ 0.5

* Significant at 0.05 level. ** Significant at 0.01 level.

Fig. 1. Metaphases from mouse bone-marrow showing: SCE's induced by (a) Normal BrdU, (b) mitomycin C, (c) acrylonitrile. Metaphases from mouse spermatocytes showing. (d) x-y univalent, (e) autosomal univalent, (f) fragment. Metaphases from mouse bone-marrow showing. (g) chromatid gap, (h) chromatid break, (i) A metaphase from spleen showing Robertsonian translocation (RT) and fragment.

Table 2. Frequency of chromosomal aberrations in primary spermatocytes of male mice induced after oral treatment with different concentrations of acrylonitrile

Treatment and doses	Harvest time (hr) after the last treatment	Total number of examined metaphases (n)	No. of abnormal metaphases	Mean % abnormal metaphases $\pm$ S.E.	No. of metaphases with different types of chromosomal aberrations ^a						
					x-yu	A-u	x-yu+A-u	Frag.	Break	Gap	Chain IV
I. Single dose treatment											
A. Control (non treated)		400	16	4 $\pm$ 0.4	11	4	—	1	—	—	—
B. Acrylonitrile 7.75 mg kg ⁻¹ b.wt.	24	400	17	4.25 $\pm$ 0.25	10	2	1	—	2	2	—
15.5 mg kg ⁻¹ b.wt.	24	400	33	8.25 $\pm$ 0.48**	25	7	—	1	—	—	—
31 mg kg ⁻¹ b.wt.	24	400	46	11.5 $\pm$ 0.87**	31	8	—	2	—	1	3+1 ⁺
II. Repeated dose treatment											
3 Successive doses											
A. Control		400	15	3.75 $\pm$ 0.48	11	3	—	—	—	1	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	43	10.75 $\pm$ 0.63**	23	7	3	3	2	2	3
5 Successive doses											
A. Control		400	17	4.25 $\pm$ 0.48	12	5	—	—	—	—	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	46	11.5 $\pm$ 0.64**	25	12	3	1	2	1	2
	7 days	400	26	6.5 $\pm$ 0.6	15	8	1	2	—	—	—
Mitomycin C											
1 mg kg ⁻¹ b.wt. (+ve Control)	24	400	75	18.75 $\pm$ 0.48	31	16	—	3	5	17	3

(n)=Number of animals per each treatment group=5, ^a x-yu=x-y univalent, A-u=autosomal univalent, Frag.=Fragment, + =one cell chain III+1, ** = Significant at 0.01 level (t-test).

Table 3. Frequency of chromosomal aberrations in mouse bone-marrow cells induced after oral treatment with different concentrations of acrylonitrile

Treatment and doses	Harvest time (hr) after the last treatment	Total number of examined metaphases (n)	No. of abnormal metaphases	Mean % abnormal metaphases $\pm$ S.E.		No. of metaphases with different types of chromosomal aberrations ^a						
				Including gaps	Excluding gaps	Chromatid gap	Chromosome gap	Break or fragment	Del.	M.A.	R.T.	Polyp.
I. Single dose treatment												
A. Control (not treated)		400	14	3.5 $\pm$ 0.29	1.5 $\pm$ 0.48	8	—	3	3	—	—	—
B. Acrylonitrile												
7.75 mg kg ⁻¹ b.wt.	24	400	33	8.25 $\pm$ 0.75**	2.5 $\pm$ 0.5	20	3	3	6	1	—	—
15.5 mg kg ⁻¹ b.wt.	24	400	47	11.9 $\pm$ 1.1*	5.75 $\pm$ 0.92**	22	2	7	11	4	—	1
31 mg kg ⁻¹ b.wt.	24	400	54	13.5 $\pm$ 0.7**	6.5 $\pm$ 0.46**	23	5	10+1 ⁺	8	4+1 ⁺⁺	2	—
II. Repeated dose treatment												
3 Successive doses												
A. Control		400	15	3.75 $\pm$ 0.47	1.25 $\pm$ 0.23	10	—	3	2	—	—	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	42	10.5 $\pm$ 0.87**	4.5 $\pm$ 0.64**	20	4	4	10	2	—	2
5 Successive doses												
A. Control		400	17	4.25 $\pm$ 0.63	1.5 $\pm$ 0.42	11	—	1	5	—	—	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	51	12.75 $\pm$ 0.55**	7.75 $\pm$ 0.7**	17	3	9	10	1	6	5
	7 days	400	20	5 $\pm$ 0.4	1.75 $\pm$ 0.4	12	1	1	6	—	—	—
Mitomycin C												
1 mg kg ⁻¹ b.wt. (+ve Control)	24	400	118	29.5 $\pm$ 0.7	21.75 $\pm$ 1.1	25	6	42	23	11	9	2

(n) = Number of animals per each treatment group = 5, *M.A. = Metaphases containing more than one type of chromosomal aberrations, R.T. = Robertsonian translocation, + = Double minutes, ++ = Quadriradial configuration. ** = Significant at the 0.01 level (t-test).

Table 4. Frequency of chromosomal aberrations in mouse spleen cells induced after oral treatment with different concentrations of acrylonitrile

Treatment and doses	Harvest time (hr) after the last treatment	Total number of examined metaphases (n)	No. of abnormal metaphases	Mean % abnormal metaphases $\pm$ S.E.		No. of metaphases with different types of chromosomal aberrations ^a						
				Including gaps	Excluding gaps	Chromatid gap	Chromosome gap	Break or fragment	Del.	M.A.	R.T.	Polyp.
I. Single dose treatment												
A. Control (not treated)		400	12	3 $\pm$ 0.4	1.75 $\pm$ 0.82	5	—	2	5	—	—	—
B. Acrylonitrile												
7.75 mg kg ⁻¹ b.wt.	24	400	27	6.75 $\pm$ 0.5**	2 $\pm$ 0.95	15	4	4	3	1	—	—
15.5 mg kg ⁻¹ b.wt.	24	400	39	9.75 $\pm$ 0.48**	5.25 $\pm$ 0.25**	15	3	10	10	1	—	—
31 mg kg ⁻¹ b.wt.	24	400	58	14.5 $\pm$ 0.86**	7.25 $\pm$ 0.63**	27	2	10	11	3	4	1
II. Repeated dose treatment												
3 Successive doses												
A. Control		400	11	2.75 $\pm$ 0.4	1.25 $\pm$ 0.9	6	—	1	4	—	—	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	51	12.75 $\pm$ 0.48**	7.5 $\pm$ 0.8**	17	4	13+1 ⁺	11	2	3	—
5 Successive doses												
A. Control		400	16	4.0 $\pm$ 0.62	1.75 $\pm$ 0.71	9	—	2	4	1	—	—
B. Acrylonitrile (7.75 mg kg ⁻¹ b.wt.)	24	400	56	14 $\pm$ 0.57**	8.75 $\pm$ 0.46**	16	5	13	12	3	7	—
	7 days	400	19	4.75 $\pm$ 0.25	2.5 $\pm$ 0.42	8	1	5	3	—	2	—
Mitomycin C												
1 mg kg ⁻¹ b.wt. (+ve Control)	24	400	106	26.5 $\pm$ 0.8	19 $\pm$ 1.1	26	4	29	29	15	3	—

(n) = Number of animals per each treatment group = 5, ^aM.A. = Metaphases containing more than one type of chromosomal aberrations, R.T. = Robertsonian translocation, ⁺ = Double minutes, ** = Significant at the 0.01 level (t-test).

quency with that of mitomycin C $1 \text{ mg kg}^{-1} \text{ b.wt.}$ (+ve control), it was found that mitomycin C induced much higher effect 11.9 ± 0.5 (Fig. 1a–c). The results indicate that acrylonitrile is weak inducer of sister chromatid exchanges in mouse bone-marrow at the low doses.

Some authors recommended the study of chromosomal aberrations in germinal cells in which the transmissible genetic damage from one generation to another takes place (William and Hsu 1980, Hassan 1997). The results in single dose oral study showed that the tested doses of acrylonitrile with $1/8$, $1/4$, $1/2 \text{ LD}_{50}$ except the lowest one were capable of inducing significant increase in the percentage of chromosomal aberrations in mouse spermatocytes (Table 2). Such percentage reached 8.25 ± 0.48 and 11.5 ± 0.87 respectively after treatment with the doses 15.5 and $31 \text{ mg kg}^{-1} \text{ b.wt.}$ as compared with 4 ± 0.4 for the control non-treated. Such increase did not exceed the value of mitomycin C (18.75 ± 0.48). Multiple treatment with the dose $7.75 \text{ mg kg}^{-1} \text{ b.wt.}$ ($1/8 \text{ LD}_{50}$) caused higher percentage of chromosomal aberrations than single treatment. In mouse spermatocytes, no other aberrations other than structural aberrations were observed (Fig. 1d–f). Univalent formation was highly increased (x–y and autosomal univalents). However, x–y univalents were much more frequent. Such a phenomenon was observed also by Imai *et al.* (1981). Ford (1969), reported that autosomal univalents may result from asynapsis (absence of zygotene pairing between homologous segments) or from desynapsis (separation due to the failure of chiasma formation). Precocious separation of x–y, or x–y dissociation has been discussed as a mechanism of male sterility from radiation and mutagenic chemicals. Also breaks, fragments and gaps were observed. Translocation in the form of chain IV was observed in low percentage after the treatment with the highest dose and the treatment with multiple administration. This result is in agreement with the finding of Leonard (1976), Brewen and Preston (1978) who reported that chemicals (*e.g.* mitomycin C, procarbazine and triethylenemelamine) induced low percentage of translocation which was recorded in high frequency after exposure to radiation (EL-Dawy 1993).

Acrylonitrile also induced significant increase in the percentage of chromosomal aberrations in bone-marrow as well as spleen cells even after excluding gaps ($p < 0.01$) except with the lowest tested dose (single oral treatment) (Tables 3, 4). The frequency of chromosomal aberrations increased as the concentration of acrylonitrile goes high. This fact indicates that the overall trend of response to the dose was significant.

Multiple treatment caused a higher percentage of chromosomal aberrations than single treatment. This may be due to the accumulation effect of acrylonitrile, since the animals in multiple treatment received 3 or 5 times more of the single treatment. Moreover chromosome aberrations returned to the normal level when mice were left to recover for 1 week after the last treatment which revealed complete elimination of acrylonitrile. Positive control data showed high frequency of chromosomal aberrations over those of the negative control and treated animals.

Concerning the different types of the aberrations observed (Fig. 1g–i), they were mainly of chromatid type (gaps, breaks, fragments, deletions). However metaphases carrying more than one type of structural chromosomal aberrations (multiple aberrations) were rare, while those with one aberration only were dominant. Robertsonian translocation (RT) appeared only with the highest tested dose of acrylonitrile and after repeated treatment. Numerical aberrations were rare and observed only in bone-marrow with repeated treatment. Accordingly the induction of chromosome aberrations following exposure to acrylonitrile revealed a potential for clastogenicity.

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