ACETAMIDE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

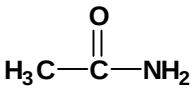
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 60-35-5

Systematic name: Acetamide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_5NO Relative molecular mass: 59.1

1.1.3 *Physical properties* (for details, see IARC, 1974)

(*a*) *Boiling-point*: 222°C

(*b*) *Melting-point*: 81°C

(*c*) *Conversion factor*: $mg/m^3 = 2.42 \times ppm$

1.2 Production and use

Acetamide has been produced commercially since the 1920s, but it is not certain that it is still in commercial use, although it was previously used as an intermediate in the synthesis of methylamine, thioacetamide, hypnotics, insecticides, medicinals and various plastics, a solvent, a soldering flux ingredient, a wetting agent and penetration accelerator for dyes, and as a plasticizer in leather, cloth and coatings (IARC, 1974).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Acetamide was tested for carcinogenicity by oral administration in rats, producing benign and malignant liver tumours (IARC, 1974).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female C57BL/6 mice were fed diets containing 1.18% or 2.36% acetamide for 12 months. In male mice treated with acetamide, an increase in the incidence of malignant lymphomas was observed (controls, 0/95; low-dose, 7/50; high-dose, 7/46; $p = 0.004$, Cochran-Armitage test for trend) (Fleischman *et al.*, 1980).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats were fed 0 or 2.36% acetamide in the diet for 12 months. Neoplastic nodules were seen in the liver in 0/50 and 1/47 control and treated males and in 0/49 and 3/48 control and treated females, respectively. Hepatocellular carcinomas were found in 0/50, 41/47, 0/49, 33/48 male controls, male treated, female controls and female treated rats, respectively. The incidence, speed of onset and frequency of metastases were greater in males than in females (Fleischman *et al.*, 1980).

In a study of hepatocellular changes induced by acetamide, 60 male Leeds strain rats were administered a diet containing 5.0% acetamide for up to 35 weeks and were then returned to a control diet for up to a further nine months. The experiment included a control group of 40 male rats. Subgroups of four rats from the treated and control groups were killed at nine days and four, 10, 26 and 35 weeks from the beginning of the experiment. Similar numbers from the treated and control groups were killed at one, four and six months and all survivors were killed at nine months after the end of treatment. All acetamide-treated rats killed at 26 weeks had neoplastic nodules of the liver. Hepatocellular carcinomas arose after cessation of treatment in 1/4 rats killed at one month, 5/10 rats killed at four months, 7/8 rats killed at six months and 4/4 rats killed at nine months (Flaks *et al.*, 1983).

3.2 Administration with known carcinogens

Rat: Acetamide was studied for initiating activity in a modified Solt-Farber system at single intraperitoneal doses of 100 and 400 mg/kg bw administered to groups of 12 male Fischer 344 rats. Two weeks after injection, all of the rats were treated with 2-acetylaminofluorene (AAF), 2 mg/kg bw, every other day for two weeks. One week after commencing the AAF treatment, partial hepatectomies were performed. Two weeks after finishing the AAF treatment, the rats were fed a diet containing 0.05% phenobarbital for two months, after which they were killed and their livers examined. A negative control group of five rats and a positive control group of four rats were included in which acetamide treatment was replaced by saline and *N*-nitrosodiethylamine (100 mg/kg bw)

treatments, respectively. γ-Glutamyltranspeptidase (γ-GT)-positive foci occurred in 0/5, 5/12 and 12/12 rats of the negative control and the 100 and 400 mg/kg bw acetamide-dosed groups, respectively. Precise quantitation of the foci was not possible. Livers with γ-GT-positive foci showed basophilia, lipidosis and periportal hypertrophy, while γ-GT-negative livers had normal morphology. It was concluded that acetamide shows properties in this system consistent with initiator activity (Dybing *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The half-life of radioactivity in blood after intravenous dosing of [^{14}C]acetamide to rats averaged 20.6 ± 0.3 h after a 10 mg/kg bw dose and 16.1 ± 1.6 h after a 50 mg/kg bw dose. The volume of distribution was about 1 mL/g, total body clearance was 0.27 mL/min and renal clearance was 0.19 mL/min. Approximately 64–72% of [^{14}C]acetamide was excreted in the urine, while only 0.5–0.8% appeared in exhaled air during the first 6 h after dosing. Thus, approximately 30% of the administered dose was not recovered and it was suggested that metabolized acetamide enters the acetate pool (Putcha *et al*., 1984).

Less than 0.07% of the recovered urinary radioactivity in rats given 100 or 1000 mg/kg bw [^{14}C]acetamide coeluted upon high-performance liquid chromatography with an *N*-hydroxyacetamide standard and this hydroxamic acid could not be detected after incubation of acetamide with rat liver microsomes and NADPH or in primary cultures of rat hepatocytes. [^{14}C]Acetamide does not bind covalently to proteins in the presence of rat liver microsomes and NADPH or cytosolic fraction, whereas hepatocyte cultures contained non-extractable radioactivity. This association was inhibited by cycloheximide to the same extent as [^{14}C]acetate incorporation into cellular proteins (Dybing *et al.*, 1987).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

During treatment with a diet containing 5.0% acetamide that led to a high incidence of hepatocellular carcinomas in male Leeds strain rats, neither necrosis nor cirrhosis occurred, in sharp contrast to the response to thioacetamide. Ultrastructural changes within hepatocytes of the acetamide-treated rats gave only minor indications of toxicity (Flaks *et al*.,

1983). The putative metabolite, *N*-hydroxyacetamide, did not cause necrosis within 24 h following a single intraperitoneal injection at doses of 100, 400, 500 and 1000 mg/kg bw, even when some rats in the latter two groups were also treated with 600 mg/kg bw of the glutathione-depleting agent, diethyl maleate, 30 min before *N*-hydroxyacetamide. Toxicity was assayed on the basis of liver morphology and plasma glutamic-oxaloacetic transaminase analysis. However, 2.5 mM *N*-hydroxyacetamide did not deplete cellular glutathione levels in primary cultures of rat hepatocytes (Dybing *et al*., 1987).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Tables 1 and 2 for references)

Acetamide does not cause differential toxicity in repair-deficient *Escherichia coli* strains, and is not mutagenic, with or without metabolic activation, in *Salmonella typhimurium* or *Saccharomyces cerevisiae* or, without metabolic activation, in *Aspergillus nidulans*. It is also not mutagenic in *Saccharomyces cerevisiae* in a host-mediated assay. In *Aspergillus nidulans*, it is not recombinogenic and does not induce chromosome malsegregation. It may weakly induce intrachromosomal recombination in *Saccharomyces cerevisiae*. In *Drosophila melanogaster*, it does not induce germ cell or somatic mutations in a number of loci. However, it weakly induces somatic mutations at the *zeste-white* locus and is weakly positive in the wing spot test. In cultured mammalian cells, it does not induce DNA strand breaks in rat hepatoma cells, gene amplification in Chinese hamster ovary CO361 cells or morphological transformation of C3H 10T½ mouse embryo cells, while it gave contradictory (probably negative) results for the induction of morphological transformation in Syrian hamster embryo cells. It does not inhibit gap-junctional communication in Chinese hamster lung V79 cells. It was marginally positive in the induction of bone-marrow micronuclei in male C57BL/6 mice in one study, but it was negative in another study at higher doses in the same species as well as in CBA male mice.

Genotoxicity studies with *N*-hydroxyacetamide, a possible metabolite of acetamide, have shown that this agent is weakly mutagenic in *Salmonella typhimurium* and induces DNA damage in a rat hepatoma cell line. However, it did not bind covalently to DNA *in vitro* and did not induce morphological transformation of Syrian hamster embryo cells *in vitro* or inhibit gap-junctional intercellular communication in Chinese hamster lung V79 cells.

Table 1. Genetic and related effects of acetamide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Salmonella typhimurium* SOS induction (*umu* test)	–	–	1670	Nakamura *et al.*, 1987
ERD, *Escherichia coli rec A* strains, differential toxicity	–	–	63 800	Hellmér & Bocsfoldi (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Dybing *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	125	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	125	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	125	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	125	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Dybing *et al*. (1987)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	125	Simmon (1979a)
SCR, *Saccharomyces cerevisiae* C658-K42, reverse mutation	–	–	16000	Morita *et al*. (1989)
SCH, *Saccharomyces cerevisiae* D3, recombination	–	–	58000	Simmon (1979b)
SCH, *Saccharomyces cerevisiae* RS112, intrachromosomal recombination	(+)	NT	40000	Schiestl *et al*. (1989)
SCH, *Saccharomyces cerevisiae* RS112, interchromosomal recombination	–	NT	40000	Schiestl *et al*. (1989)
ANN, *Aspergillus nidulans*, chromosome malsegregation	–	NT	40000	Crebelli *et al*. (1986)
ANF, *Aspergillus nidulans*, forward mutation	–	NT	40000	Crebelli *et al*. (1986)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		50000 ppm feed	Valencia *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		50000 ppm inj.	Valencia *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMM, *Drosophila melanogaster*, somatic mutation/recombination $(w^i)_4$	–		2950 feed	Batiste-Alentorn *et al*. (1994)
DMM, *Drosophila melanogaster*, somatic mutation/recombination $(w^i)_4$	–		4720 feed	Consuegra *et al*. (1996)
DMM, *Drosophila melanogaster*, somatic mutation (*zeste-white* locus)	(+)		590 feed	Batiste-Alentorn *et al*. (1991)
DMM, *Drosophila melanogaster*, somatic mutation	–		4500 feed	Mitchell *et al*. (1981)
DMM, *Drosophila melanogaster*, somatic mutation	–		590 feed	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, somatic mutation and recombination test (SMART), wing spot test	(+)		2950 feed	Batiste-Alentorn *et al*. (1995)
DIA, DNA strand breaks, rat hepatoma cells *in vitro*	–	NT	14 775	Dybing *et al*. (1987)
TCM, Cell transformation C3H 10T½ mouse embryo *in vitro*	–	NT	NG	Patierno *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	(+)	NT	NG[c]	Pienta *et al*. (1977)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	(+)	NT	1000[c]	Amacher & Zelljadt (1983)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	–	NT	5000	Dybing *et al*. (1987)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells *in vitro*	–	NT	5910	Dybing *et al*. (1987)
SV40 DNA amplification, Chinese hamster ovary CO631 cells *in vitro*	+	NT	0.59	Fahrig & Steinkamp-Zucht (1996)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D3 in Swiss Webster mice, forward mutation	–		1000 ip × 1	Simmon *et al*. (1979)
MVM, Micronucleus test, male C57BL/6 mouse bone marrow *in vivo*	+		200 po × 2	Chieli *et al*. (1987)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, male CBA mouse bone marrow *in vivo*	–		5000 po × 1	Mirkova (1996)
MVM, Micronucleus test, male and female C57BL/6 mouse bone marrow *in vivo*	–		5000 po × 1	Mirkova (1996)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, peritoneal; po, oral
[c] No indication of the dose–response

Table 2. Genetic and related effects of *N*-hydroxyacetamide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	1000	Dybing *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	500	Dybing *et al.* (1987)
DIA, DNA strand breaks, cross-links or related damage, Reuber rat hepatoma cells *in vitro*	(+)	NT	750	Dybing *et al.* (1987)
TIH, Cell transformation, Syrian hamster embryo cells *in vitro*	–	–	50	Dybing *et al.* (1987)
ICR, Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	–	–	225	Dybing *et al.* (1987)

[a] (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

5. Evaluation

No epidemiological data relevant to the carcinogenicity of acetamide were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of acetamide.

Overall evaluation

Acetamide is *possibly carcinogenic to humans (Group 2B).*

6. References

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