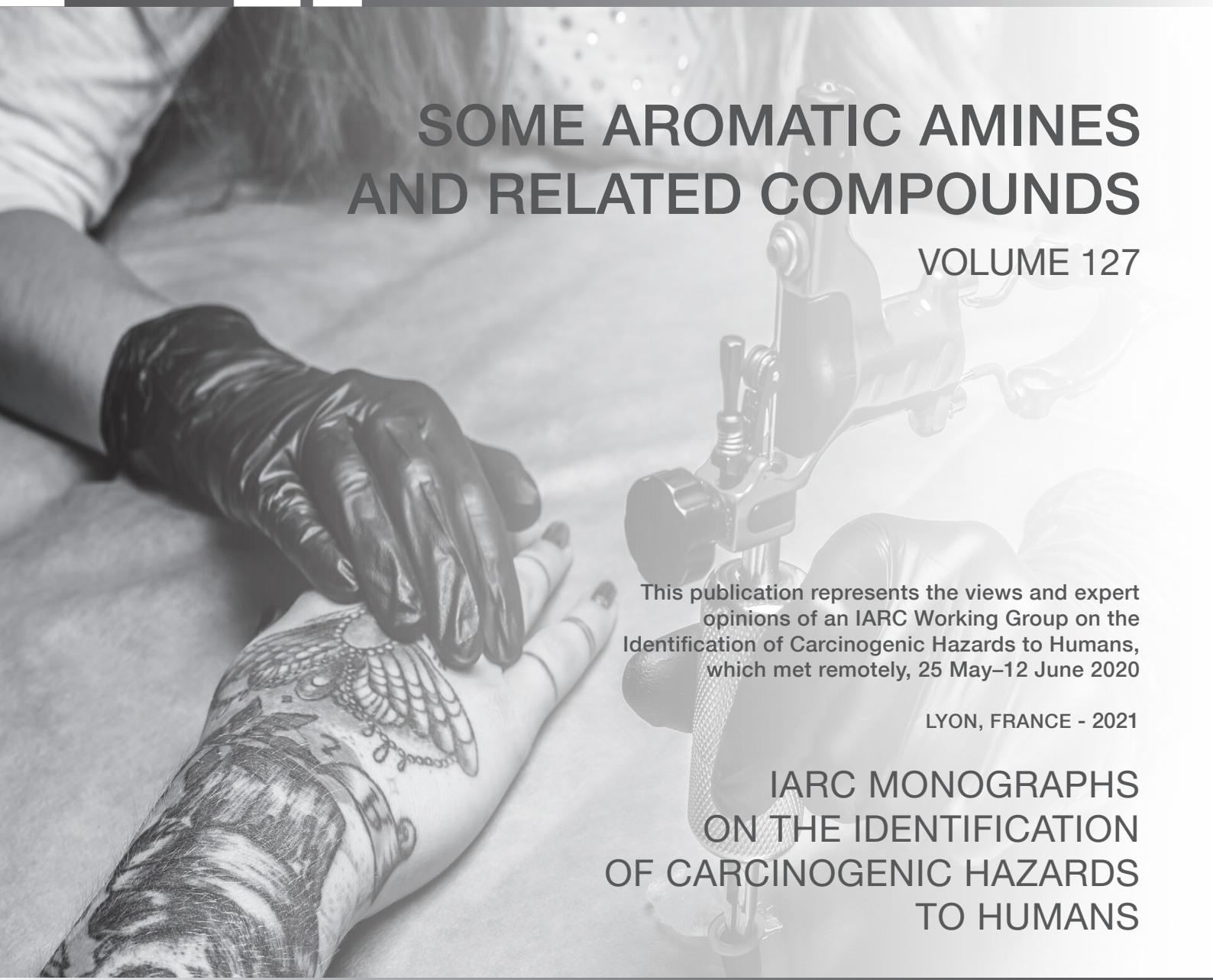
IARC MONOGRAPHS



SOME AROMATIC AMINES AND RELATED COMPOUNDS

VOLUME 127

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International Agency for Research on Cancer

World Health Organization

CUPFERRON

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

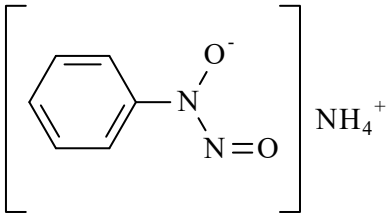
Chem. Abstr. Serv. Reg. No.: 135-20-6

EC No.: 201-183-2

IUPAC systematic name: ammonium 2-oxo-1-phenylhydrazinolate

Synonyms and abbreviations: cupferron; hydrogen cupferron; *N*-nitroso-*N*-phenylhydroxylamine; *N*-nitroso-*N*-phenylhydroxylamine ammonium salt; azanium *N*-oxido-*N*-phenylnitrous amide; ammonium *N*-nitroso-*N*-oxidoaniline; tongtielin; nitrosophenylhydroxylamine azamethane.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_9N_3O_2$

Relative molecular mass: 155.15 (NTP, 2016; ChemSpider, 2020; NCBI, 2020).

1.1.3 Chemical and physical properties of the pure substance

Description: cupferron appears as light yellow or cream-coloured crystals or a brown crystalline solid (NCBI, 2020)

Boiling point: 278.9 °C (Chemical Book, 2017)

Melting point: 163.5 °C (NCBI, 2020)

Flash point: not available; probably combustible (NCBI, 2020)

Density: 1.3092 g/cm^3 (Chemical Book, 2017)

Vapour pressure: 6.29×10^{-5} mm Hg [0.0084 Pa] at 25 °C (NTP, 2016)

Solubility: soluble in water, alcohol, and ether (NTP, 2016)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, –1.73 (NTP, 2016).

1.2 Production and use

1.2.1 Production process

Cupferron, the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine, is prepared by the reaction of phenylhydroxylamine with a nitrite source (NCBI, 2020).

1.2.2 Production volume

Cupferron is listed as an existing substance by international substance registries such as the European Chemicals Agency, the

National Industrial Chemicals Notification and Assessment Scheme, and the United States Environmental Protection Agency (US EPA), but no additional information was available to the Working Group (New Jersey Department of Health and Senior Services, 2001; ECHA, 2019; NICNAS, 2019; OECD, 2019; US EPA, 2019a).

Cupferron is currently not on the United States Toxic Substances Control Act (TSCA) Chemical Data Reporting (CDR) inventory. This information is collected every 4 years from manufacturers and importers when production volumes for the chemical are 25 000 pounds [11 300 kg] or greater in any reporting year. In 2020, cupferron was available from approximately 30 suppliers, mainly based in China and the USA (Chemical Register, 2020). The NTP Report on Carcinogens (NTP, 2016) provides additional historical context, indicating that cupferron was produced by one manufacturer in east Asia and four manufacturers in India, and was available from 28 suppliers, including 17 suppliers in the USA in 2009. Reports submitted to the US EPA under the TSCA inventory requirements from 1986 to 2002 (except in 1994) indicated that USA production plus imports of cupferron totalled about 10 000–500 000 pounds [4500–230 000 kg] (NCBI, 2020).

1.2.3 Uses

Cupferron is soluble in water and alcohol, and as a common reagent can be used to separate metals such as copper, iron, tin, vanadium, and thorium from other metals (NTP, 2016; NCBI, 2020). In analytical laboratories, cupferron is a reagent used for quantitative determination of vanadates and titanium, and the colorimetric determination of aluminium (NTP, 2016; Grabarczyk & Adamczyk, 2017; NCBI, 2020). [The Working Group noted that, despite the sizable global production volume of cupferron, little to no information was available on the distribution and levels of use across different industries and occupations.]

1.3 Measurement and analysis

There are no reported methods for chemical analysis of cupferron in exposure-relevant matrices. The purity of cupferron reagent is quantified by chemical manufacturers using high-performance liquid chromatography coupled to ultraviolet detection (HPLC-UV) at λ_{max} = 282 nm (VWR, 2020).

1.4 Occurrence and exposure

No Toxic Release Inventory (TRI) data required by the TSCA were available after 2001 for cupferron reported to be disposed of or otherwise released on-site and off-site in the USA. No recent data on occupational or general population exposures to cupferron were available. The primary routes of potential human exposure to cupferron are ingestion and inhalation of the dust of the dry salt. Dermal and eye contact are secondary routes of potential exposure (NTP, 2016; NCBI, 2020). An earlier report by the National Cancer Institute (NCI) noted that the potential for exposure appeared to be greatest among individuals engaged in analytical or research studies involving the use of cupferron. Workers may also potentially be exposed during manufacturing processes (NCI, 1978). [The Working Group noted that original references for these statements could not be identified.]

The United States National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that 136 chemical technicians in the primary metal industries were potentially exposed to cupferron (NOES, 1990).

1.5 Regulations and guidelines

Cupferron is reportable under the Emergency Planning and Community Right-To-Know Act (Section 313), or the US EPA's TRI (US EPA, 2019a), with a "de minimus" concentration of 0.1%. This "de minimus" concentration refers to the presence of cupferron in a mixture of chemicals in a regulated facility at a concentration below 0.1% of the mixture (US EPA, 2017).

The California Office of Environmental Health Hazard Assessment (OEHHA) "safe harbour" level (no-significant-risk level) is 3 µg/day (OEHHA, 1992).

No occupational exposure limits have been established for cupferron. In Germany, the first general administrative regulation for the Federal Emissions Control Act of 2002 against harmful effects from air pollution sets the maximum allowed mass concentration in exhaust gas at 20 mg/m^3. No other exposure limits, guidelines, or reference values were identified internationally (NTP, 2016; IFA, 2020).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (feed)

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks), received feed containing cupferron (purity, approximately 93%) at time-weighted average concentrations of 0.2% (lower dose) or 0.4% (higher dose), 7 days per week, for 78 weeks, followed by an additional observation period of 17–18 weeks. [Lower-dose and higher-dose mice were treated with 0.3% or 0.6%, respectively, for 35 weeks, then 0.1% or 0.2%, respectively, for 43 weeks, due to high mortality and excess decrease in mean body weight.] Groups of 50 male and 50 female control mice (age, 11 weeks) received feed alone for 98 weeks (NCI, 1978). There was a positive dose-related trend in mortality that was significant for both male and female mice. In males, survival to the end of the study was 42/50 (controls), 29/50 (lower dose), and 20/50 (higher dose); however, survival for at least 75 weeks was 49/50, 44/50, and 31/50, respectively. In females, survival to the end of the study was 40/50, 34/50, and 29/50, respectively. [Sufficient numbers of male and female mice survived long enough to evaluate the risk of late-developing tumours.] There was a significant dose-related decrease in mean body weight in treated male and female mice compared with controls over the course of the study. Full histopathology was performed on major tissues, organs, and gross lesions taken from mice that were killed and from mice found dead.

In male B6C3F$_1$ mice at the higher dose, there was a significant increase ($P = 0.013$) in the incidence of haemangiosarcoma of the circulatory system [not otherwise specified (NOS), mostly originating in the spleen] compared with male controls, with a significant positive trend ($P = 0.008$). There was also a significant positive trend ($P = 0.028$) and increase ($P < 0.036$) in the incidence of adenoma (NOS) of the Harderian gland at the higher dose compared with male controls. In female mice, there was a significant positive trend [$P < 0.05$] and significant increase in the incidence of haemangioma or haemangiosarcoma (combined) of the circulatory system at the lower ($P = 0.003$) and higher dose ($P = 0.044$) compared with female controls. There was also a significant positive trend ($P = 0.038$) and significant increase ($P = 0.044$) in the incidence of haemangiosarcoma of the circulatory system in females at the higher dose compared

Table 3.1 Studies of carcinogenicity with cupferron in mice and rats

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M) 6 wk (controls, 11 wk) 95–98 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.2%, 0.4%, 7 days/wk for 78 wk 50, 50, 50 42, 29, 20	*Circulatory system*: haemangiosarcoma 1/50, 3/45, 7/40*	$P = 0.008$, Cochran–Armitage trend test; *$P = 0.013$, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate. Principal limitations: TWA doses; mice were treated with 0.3% (lower dose) or 0.6% (higher dose) for 35 wk, then 0.1% (lower dose) or 0.2% (higher dose) for 43 wk, due to high mortality and excess mean body-weight decrease. A positive dose-related trend in mortality was significant for males ($P < 0.001$). Five mice in the control group were killed at wk 80 and five mice at the higher dose were killed at wk 78. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found.
		Harderian gland: adenoma, NOS 0/50, 3/45, 4/40*	$P = 0.028$, Cochran–Armitage trend test; *$P < 0.036$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (F) 6 wk (controls, 11 wk) 95–98 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.2%, 0.4%, 7 days/wk, for 78 wk 50, 50, 50 40, 34, 29	*Circulatory system* Haemangiosarcoma 1/50, 5/47, 6/46*	$P = 0.038$, Cochran–Armitage trend test; *$P = 0.044$, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate. Principal limitations: TWA doses; mice were treated with 0.3% (lower dose) or 0.6% (higher dose) for 35 wk, then 0.1% (lower dose) or 0.2% (higher dose) for 43 wk, due to high mortality and excess mean body-weight decrease. A positive dose-related trend in mortality was significant for females ($P = 0.009$). Five mice in the control group were killed at wk 80 and five mice at the higher dose were killed at wk 78. Incidence of Zymbal gland squamous cell carcinoma or sebaceous adenocarcinoma in historical controls was 0/275. Statistical analyses were performed on mice that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found.
		Haemangioma or haemangiosarcoma (combined) 1/50, 10/47*, 6/46**	[$P < 0.05$, Cochran–Armitage trend test]; *$P = 0.003$, Fisher exact test; **$P = 0.044$, Fisher exact test	
		Lung Bronchioloalveolar adenoma 1/50, 8/45*, 8/46**	[$P = 0.022$, Cochran–Armitage trend test]; *[$P = 0.012$, Fisher exact test]; **[$P = 0.013$, Fisher exact test]	
		Bronchioloalveolar carcinoma 3/50, 3/45, 1/46	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 4/50, 11/45*, 9/46	*$P = 0.027$, Fisher exact test	
		Liver Hepatocellular carcinoma 2/49, 9/46*, 13/45**	$P = 0.001$, Cochran–Armitage trend test; *$P = 0.019$, Fisher exact test; **$P = 0.001$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined) 2/49, 12/46*, 16/45**	$P < 0.001$, Cochran–Armitage trend test; *$P = 0.002$, Fisher exact test; **$P < 0.001$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (F) 6 wk (controls, 11 wk) 95–98 wk NCI (1978) (cont.)		*Harderian gland*: adenoma, NOS 0/50, 2/47, 6/46*	*P* = 0.006, Cochran–Armitage trend test; **P* = 0.010, Fisher exact test	
		Zymbal gland: squamous cell carcinoma or sebaceous adenocarcinoma (combined) 0/50, 0/47, 3/46	*P* = 0.033, Cochran–Armitage trend test	
Rat, F344 (M) 6 wk (controls, 16 wk) 97–110 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.15%, 0.30%, 7 days/wk, for 78 wk 50, 50, 49 32, 0, 0	*Circulatory system*: haemangiosarcoma 0/50, 38/49*, 35/44*	*P* < 0.001, Cochran–Armitage trend test; **P* < 0.001, Fisher exact test	Principal strengths: use of males and females; adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate; the schedule of exposure was adequate. Principal limitations: one rat was removed (wrong sex) from the group of males at the higher dose. There was a significant (*P* < 0.001) positive association between dosage and mortality in males. In historical controls, 11/250 (4%) of the untreated male F344 rats had subcutaneous fibroma. Five males in the control group were killed at wk 78. Statistical analyses were performed on rats that survived at least 52 wk, unless a tumour was observed before wk 52: comparisons were based on animals that survived at least as long as the animal in which the tumour was found. Further subtyping of liver neoplastic nodules was not possible due to the lack of original data for review.
		Liver Hepatocellular carcinoma 0/49, 8/48*, 4/43**	[*P* = 0.011, Cochran–Armitage trend test]; **P* = 0.003, Fisher exact test; ***P* = 0.044, Fisher exact test	
		Hepatocellular carcinoma or neoplastic nodules (combined) 0/49, 12/48*, 5/43**	*P* = 0.048, Cochran–Armitage trend test; **P* < 0.001, Fisher exact test; ***P* = 0.020, Fisher exact test	
		Forestomach Squamous cell carcinoma		

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (M) 6 wk (controls, 16 wk) 97–110 wk NCI (1978) (cont.)		0/49, 19/48*, 17/38*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined)		
		0/49, 32/48*, 24/38*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Body cavities: malignant mesothelioma or mesothelioma (NOS) (combined)		
		0/50, 5/49*, 1/44	*$P = 0.027$, Fisher exact test	
		Subcutaneous tissue: fibroma		
		1/50, 15/49*, 5/44	[$P = 0.04$, Cochran–Armitage trend-test]; *$P < 0.001$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344 (F) 6 wk (controls, 16 wk) 106–110 wk NCI (1978)	Oral Purity, ~93% Feed 0, 0.15%, 0.30%, 7 days/wk, for 78 wk 50, 50, 50 36, 8, 2	*Circulatory system*: haemangiosarcoma 0/49, 28/45*, 37/47*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	Principal strengths: adequate number of animals used, randomly allocated in groups; the duration of exposure and observation was adequate; the schedule of exposure was adequate; use of males and females. There was a significant ($P < 0.001$) positive association between dosage and mortality in females. Five female controls were killed at wk 78. Statistical analyses were performed on animals that survived at least 52 wk, unless a tumour was observed before wk 52; comparisons were based on animals that survived at least as long as the animal in which the tumour was found. Further subtyping of liver neoplastic nodules was not possible due to the lack of original data for review.
		Liver Hepatocellular carcinoma 1/48, 24/44*, 10/44**	$P = 0.012$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test; **$P = 0.002$, Fisher exact test	
		Hepatocellular carcinoma or neoplastic nodules (combined) 1/48, 26/44*, 12/44*	$P = 0.004$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Forestomach Squamous cell carcinoma 0/49, 14/43*, 22/43*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	
		Squamous cell papilloma or carcinoma (combined) 0/49, 19/43*, 24/43*	$P < 0.001$, Cochran–Armitage trend test; *$P < 0.001$, Fisher exact test	

F, female; M, male; NOS, not otherwise specified; NS, not significant; TWA, time-weighted average; wk, week.

with controls. There was a significant positive trend [$P = 0.022$] and significant increase in the incidence of bronchioloalveolar adenoma in females at the lower [$P = 0.012$] and higher dose [$P = 0.013$] compared with controls (controls, 1/50; lower dose, 8/45; higher dose, 8/46). There was a small but significant increase ($P = 0.027$) in the incidence of bronchioloalveolar adenoma or carcinoma (combined) in females at the lower dose compared with controls (controls, 4/50; lower dose, 11/45, higher dose, 9/46); however, the increase in the group at the higher dose and the trend were not significant, and the incidence of bronchioloalveolar carcinoma was not significantly increased in treated female mice (controls, 3/50; lower dose, 3/45; higher dose, 1/46) [The Working Group considered that the increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) was not related to the treatment]. There was a significant increase ($P \leq 0.019$) in the incidence of hepatocellular carcinoma in females at the lower and higher dose, with a significant positive trend ($P = 0.001$). There was also a significant increase ($P \leq 0.002$) in the incidence of hepatocellular adenoma or carcinoma (combined) in females at the lower and higher dose, with a significant positive trend ($P < 0.001$), compared with controls. In female mice, a significant increase ($P = 0.010$) in the incidence of Harderian gland adenoma (NOS), with a significant positive trend ($P = 0.006$), was observed at the higher dose compared with controls. In females, there was a significant positive trend ($P = 0.033$) in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland compared with controls (NCI, 1978).

[The Working Group noted the adequate number of animals used, random allocation in groups, and the use of males and females. The duration of exposure and observation was adequate.]

3.2 Rat

Oral administration (feed)

Groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing cupferron (purity, approximately 93%) at a concentration of 0.15% (lower dose) or 0.30% (higher dose), 7 days per week, for 78 weeks and then untreated for an additional observation period of 19–28 weeks. Control groups of 50 males and 50 females (age, 16 weeks) received feed alone for 110 weeks (NCI, 1978). One rat was removed (wrong sex) from the group of males at the higher dose. There was a significant positive trend in mortality in treated male and female rats. The median survival of male rats was 63 weeks in the group at the higher dose (all rats had died by week 98), and 84 weeks in the group at the lower dose (all rats had died by week 105). In the control group, 64% (32/50) of the males survived until the end of the study. The median survival of female rats at the higher dose was 68 weeks, with two rats surviving until the end of the study. The median survival of female rats at the lower dose was 91 weeks, with eight rats surviving until the end of the study. In the control group, 72% (36/50) of the female rats survived until the end of the study. [The early mortality in treated males and females may have resulted from an increased incidence of haemangiosarcoma as early as week 42 in males and week 43 in females.] Full histopathology was performed on major tissues, organs, and gross lesions taken from rats that were killed and rats that were found dead.

In male and female rats, there was a significant increase ($P < 0.001$), with a significant positive trend ($P < 0.001$), in the incidence of haemangiosarcoma of the circulatory system (mostly originating in the spleen) at the lower and higher dose compared with controls. In male rats, the incidence of hepatocellular carcinoma or neoplastic nodules (combined) of the liver [further subtyping of the liver neoplastic

nodules was not possible due to the lack of original data for review] was significantly increased, with a significant positive trend ($P = 0.048$), at the lower dose ($P < 0.001$) and at the higher dose ($P = 0.020$); in female rats, the incidence was significantly increased, with a significant positive trend ($P = 0.004$), at the lower dose ($P < 0.001$) and at the higher dose ($P < 0.001$), compared with controls. There was also a significant positive trend [$P \leq 0.012$] and significant increase in the incidence of hepatocellular carcinoma at the lower ($P \leq 0.003$) and higher ($P \leq 0.044$) doses in male and female rats. In males and in females, there was a significant positive trend ($P < 0.001$) and a significant increase ($P < 0.001$) in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach, and of squamous cell carcinoma of the forestomach at the lower and higher doses compared with controls. There was a significant increase ($P = 0.027$) in the incidence of malignant mesothelioma or mesothelioma (NOS) (combined) of the body cavities in male rats at the lower dose compared with controls. In male rats, the incidence of fibroma of the subcutaneous tissue was significantly increased ($P < 0.001$) at the lower dose compared with controls, with a significant positive trend [$P = 0.04$].

Regarding non-neoplastic lesions, basophilic foci and clear cell foci of the liver were observed only in treated male and female rats, and the incidence of focal and diffuse basal cell hyperplasia of the forestomach was significantly increased in treated male and female rats (NCI, 1978). [Hyperplasia of the forestomach may be a pre-neoplastic lesion, based on the presence of forestomach tumours.]

[The Working Group noted the adequate number of animals used, random allocation in groups, and the use of males and females. The duration of exposure and observation was adequate, and the schedule of exposure was adequate.]

3.3 Synthesis

In one study in male and female B6C3F$_1$ mice treated by oral administration (in feed), cupferron caused a significant increase, with a significant positive trend, in the incidence of haemangiosarcoma of the circulatory system and adenoma (NOS) of the Harderian gland in males; and of haemangiosarcoma and haemangioma or haemangiosarcoma (combined) of the circulatory system, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined), bronchioloalveolar adenoma, and adenoma (NOS) of the Harderian gland in females. There was also a significant positive trend in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland in females (NCI, 1978).

In one study in male and female Fischer 344 rats treated by oral administration (in feed), cupferron caused a significant increase, with a significant positive trend, in the incidence of haemangiosarcoma of the circulatory system, hepatocellular carcinoma and hepatocellular carcinoma or neoplastic liver nodules (combined), and squamous cell carcinoma and squamous cell papilloma or carcinoma (combined) of the forestomach in males and females; and of fibroma of the subcutaneous tissue in males. There was also a significant increase in the incidence of malignant mesothelioma or mesothelioma (NOS) (combined) of the body cavities in male rats (NCI, 1978).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

No information on the absorption, distribution, metabolism, or excretion of cupferron in biological systems was available to the Working Group.

4.2 Evidence relevant to the key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether cupferron is genotoxic, and alters cell proliferation, cell death or nutrient supply. For the evaluation of the other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is genotoxic

(a) Humans

No data in exposed humans were available to the Working Group.

In a test for inhibition of DNA synthesis in cultured HeLa cells (a human cervical carcinoma cell line), cupferron (in the absence of exogenous metabolic activation) inhibited replicative DNA synthesis (Heil & Reifferscheid, 1992).

(b) Experimental systems

See Table 4.1.

(i) Cytogenetic effects in mammalian cells

In tests for cytogenetic effects in Chinese hamster ovary (CHO) cells, cupferron caused significant increases in the frequency of chromosomal aberrations in two studies and sister-chromatid exchange in two studies in a concentration-related manner in the presence, but not in the absence, of rat liver S9 (NTP, 1989a, b). [The Working Group noted that the highest concentrations tested in the absence of S9 were 10 times lower than the lowest concentrations tested in the presence of S9.]

*(ii) Gene mutation and SOS/*umu *genotoxicity in bacteria*

Cupferron gave generally positive results in *Salmonella typhimurium* strain TA1538 (in the absence of S9, and in the presence of mouse, rat, or hamster liver S9) and in strain TA98 (in the absence of S9 and in the presence of rat or hamster liver S9) (Dunkel et al., 1985; NTP, 1989c; Zeiger et al., 1992). Positive mutagenic responses were obtained in *Escherichia coli* in the presence of either rat or hamster S9 (Dunkel et al., 1985). Cupferron gave negative results in tests in various other *Salmonella typhimurium* strains including TA97, TA98, TA100, TA1535, and TA1537 (Dunkel et al., 1985; NTP, 1989c; Zeiger et al., 1992).

Cupferron gave positive results in the SOS/*umu* genotoxicity assay in *Salmonella typhimurium* TA1535/pSK1002 and NM2009 strains (Reifferscheid & Heil, 1996).

Contrera et al. (2005) used electrotopological E-state indices and MDL quantitative structure–activity relationship (QSAR) software to predict the mutagenic potential of cupferron. The probability that cupferron is a member of the high-risk class was predicted to be 1 (from a range of 0 to 1).

4.2.2 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Male and female Fischer 344 rats receiving feed containing cupferron at a concentration of 0.15% or 0.3% for 78 weeks exhibited an increased incidence of focal and diffuse basal cell hyperplasia in the forestomach, a site at which treatment-related increases in tumour incidence were also observed (NCI, 1978).

4.3 Data relevant to comparisons across agents and end-points

The analysis of the in vitro bioactivity of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity

Table 4.1 Genetic and related effects of cupferron in experimental systems in vitro

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
CHO cells	Chromosomal aberrations		+	541 µg/mL	Purity, > 98 RLI; LEC was the lowest concentration tested	NTP (1989a)
CHO cells	Chromosomal aberrations		+	2000 µg/mL	Purity, > 98%; RLI; LEC was the lowest concentration tested	NTP (1989a)
CHO cells	Chromosomal aberrations	–		54 µg/mL	Purity, > 98%	NTP (1989a)
CHO cells	Sister-chromatid exchange		+	833 µg/mL	Purity, > 98%; RLI	NTP (1989b)
CHO cells	Sister-chromatid exchange		+	1500 µg/mL	Purity, > 98%; RLI; LEC was the lowest concentration tested	NTP (1989b)
CHO cells	Sister-chromatid exchange	–		83 µg/mL	Purity, > 98%	NTP (1989b)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; three positive findings with RLN, MLN, and MLI	Dunkel et al. (1985)
Salmonella typhimurium TA1538	Reverse mutation	±		1000 µg/plate	Purity, > 98%	Dunkel et al. (1985)
Salmonella typhimurium TA98	Reverse mutation	±	+	1000 µg/plate, –S9; 333 µg/plate, +S9	Purity, > 98%; one positive with HLN	Dunkel et al. (1985)
Salmonella typhimurium TA100, TA1535, TA1537	Reverse mutation	–	–	1000 µg/plate	Purity, > 98%; all negative	Dunkel et al. (1985)
Salmonella typhimurium TA1538	Reverse mutation	+		100 µg/plate	Purity, > 98%	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	166 µg/plate	Purity, > 98%; 5% HLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; 10% or 30% HLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	100 µg/plate	Purity, > 98%; 5% RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1538	Reverse mutation		+	333 µg/plate	Purity, > 98%; 10% RLI	NTP (1989c); Zeiger et al. (1992)

Table 4.1 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA1538	Reverse mutation		–	666 µg/plate	Purity, > 98%; 30% RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA97	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA98	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 10% HLI or RLI; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA98	Reverse mutation		+	333 µg/plate	Purity, > 98%; 5% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA100, TA1535	Reverse mutation	–	–	666 µg/plate	Purity, > 98%; 30% HLI or RLI	NTP (1989c); Zeiger et al. (1992)
Salmonella typhimurium TA1535/pSK1002 and NM2009 (SOS/*umu* test)	DNA damage	+	+	NR	Purity, NR; RLI	Reifferscheid & Heil (1996)
Escherichia coli WP2*uvrA*	Reverse mutation		+	333 µg/mL	Purity, > 98%; two positive findings with RLI, HLI	Dunkel et al. (1985)
Escherichia coli WP2*uvr*A	Reverse mutation	–	–	1000 µg/mL	Purity, > 98%; with RLN, MLN, MLI, HLN	Dunkel et al. (1985)

CHO, Chinese hamster ovary; HIC, highest ineffective concentration; HLI, hamster liver induced S9; HLN, hamster liver S9; LEC, lowest effective concentration; MLI, mouse liver induced S9; MLN, mouse liver S9; NR, not reported; RLI, rat liver induced S9; RLN, rat liver S9; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study).

Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Of the 31 ToxCast/Tox21 tests in which cupferron was active, many assay end-points are indicative of or associated with nuclear receptor binding or activation, or DNA binding. The half-maximal activity concentration (AC_{50}) values for cupferron in these assays typically ranged from 27 to 70 μM and were orders of magnitude higher than the cupferron concentration identified as the cytotoxic limit (US EPA, 2019b). The Tox21 programme reported quality control (QC) grades for the two cupferron samples tested. The QC grade for sample Tox21_201493 was "F: Caution, incorrect MW [molecular weight]; Biological activity unreliable" at T0 (the beginning of testing) and "Still under analysis" at T4 (the end of the four-month testing period). The QC grade for the other sample (Tox21_302952) was "F: Caution, incorrect MW; Biological activity unreliable" at T0 and "Fns: No sample detected; Biological activity unreliable" at T4 (NCATS, 2021). [The Working Group considered that the Tox21 tests of cupferron were non-informative, based on QC information on the substance tested in those assays. Interpretation of the ToxCast results was considered highly uncertain, based on quality control concerns regarding the substance tested, as well as observations that assay AC_{50} values exceeded the cytotoxic limit by orders of magnitude.]

5. Summary of Data Reported

5.1 Exposure characterization

Cupferron is the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine. It is a reagent used to separate metals such as copper, iron, tin, vanadium, and thorium from other metals. In 2020, cupferron was available from approximately 30 suppliers, mainly based in China and the USA. No information on uses outside the USA was identified. While there are limited data available publicly, cupferron is not listed under the requirements of the United States Environmental Protection Agency Toxic Substances Control Act Chemical Data Reporting rule, which indicates that it is produced or imported at levels below 11 300 kg/year in the USA at a single site. Occupational exposures to cupferron may occur through ingestion and inhalation of the dust of the dry salt. No recent data on occupational or general population exposures to cupferron were identified. No specific occupational or other exposure limits, guidelines, or reference values were found internationally.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Cupferron caused an increased incidence of malignant neoplasms in two species.

In $B6C3F_1$ mice, cupferron administered orally (in feed) in one study caused an increase in the incidence of haemangiosarcoma of the circulatory system in males and females, and of hepatocellular carcinoma in females. A positive trend in the incidence of squamous cell carcinoma or sebaceous adenocarcinoma (combined) of the Zymbal gland was also observed in female mice.

In Fischer 344 rats, cupferron administered orally (in feed) in one study caused an increase in the incidence of haemangiosarcoma of the circulatory system, hepatocellular carcinoma, and squamous cell carcinoma of the forestomach in males and females, and of malignant mesothelioma or mesothelioma (not otherwise specified) (combined) of the body cavities in males.

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, or excretion in humans or experimental animal systems in vivo were available.

There is consistent and coherent evidence that cupferron exhibits key characteristics of carcinogens in experimental systems. No data in humans or in experimental animals in vivo were available; however, consistent findings were seen across several test systems from different species. Cupferron is genotoxic. In the one available study in cultured human cells, cupferron inhibited DNA synthesis, an indirect indication of DNA damage, in human cervical carcinoma (HeLa) cells. In other mammalian systems in vitro, it is clastogenic in Chinese hamster ovary cells, inducing chromosomal aberrations and sister-chromatid exchanges in the presence of S9. In bacteria, cupferron causes DNA damage as shown by positive responses in the SOS/*umu* assay for DNA damage with or without rat liver S9. It is also a gene mutagen in bacteria, notably when metabolically activated by S9 derived from different rodent species. Cupferron was mutagenic in the frameshift *Salmonella typhimurium* strain TA1538 and its derivative TA98 with or without S9 derived from rat, mouse, and hamster. It was also mutagenic in *Escherichia coli* WP2 *uvrA* with S9 derived from rat and from hamster (but not from mouse). Quantitative structure-activity relationship modelling analyses predicted the mutagenic potential of cupferron.

Cupferron increased the incidence of focal and diffuse basal cell hyperplasia in the forestomach in male and female rats exposed chronically via the feed. No data on hyperplasia were available in mice exposed chronically.

Tests of cupferron in the assay battery of the Tox21 and ToxCast research programmes were uninformative.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of cupferron.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that cupferron exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

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

6.5 Rationale

The Group 2B evaluation for cupferron is based on *sufficient evidence* of cancer in experimental animals, and on *strong* mechanistic evidence. The evidence on cancer in humans was *inadequate* as no data were available. The *sufficient evidence* of carcinogenicity in experimental animals is based on an increased incidence of malignant neoplasms in two species. There is also *strong evidence* in experimental systems that cupferron exhibits key characteristics of carcinogens. Cupferron is genotoxic.

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