AMSACRINE

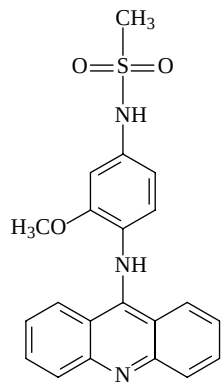
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 51264-14-3
Chem. Abstr. Name: *N*-[4-(9-Acridinylamino)-3-methoxyphenyl]methanesulfonamide
IUPAC Systematic Name: 4′-(9-Acridinylamino)methanesulfon-*meta*-anisidide
Synonyms: Acridinylanisidide; *meta*-AMSA; *meta*-Amsacrine
[Note: Amsacrine was incorrectly referred to as AMSA in some early reports (Cain & Atwell, 1974). AMSA has an -OH instead of an -OCH_3 at the 3-position of the anilino ring.]

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{21}H_{19}N_3O_3S$ Relative molecular mass: 393.47

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Yellow crystalline powder (Parke-Davis Canada, 1984)
(*b*) *Melting-point*: 230–240 °C (Parke-Davis Canada, 1984)
(*c*) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported (Dubicki *et al*., 1981).

(*d*) *Solubility*: Insoluble in water (< 1.0 mg/mL); slightly soluble in chloroform, ethanol and methanol (National Cancer Institute, 1992)
(*e*) *Reactivity*: Incompatible with saline solutions; 5% dextrose is the only recommended infusion fluid; may react with certain plastic syringes (Thomas, 1998)

1.1.4 *Technical products and impurities*

Three intravenous formulations have been used in clinical studies: amsacrine lactate, amsacrine lactate plus anhydrous *N*,*N*-dimethylacetamide and amsacrine gluconate (Hornedo & Van Echo, 1985; Louie & Issell, 1985). Amsacrine is formulated as two sterile liquids in separate ampoules, one containing 75 mg of the drug in 1.5 mL *N*,*N*-dimethylacetamide, the other containing 13.5 mL of 0.0353 mol/L lactic acid. On mixing, the resulting solution contains 5 mg/mL of amsacrine. Amsacrine is typically used in combination with other antileukaemic agents, including cytarabine, thioguanine, 5-azacytidine, vincristine and prednisone (Gennaro, 1995; Editions du Vidal, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998).

Trade names for amsacrine include Amekrin, AMSA P-D, Amsacrina, Amsacrine, Amsidil, Amsidine, Amsidyl and Lamasine (Swiss Pharmaceutical Society, 1999).

1.1.5 *Analysis*

Amsacrine has been determined in plasma by gas chromatography combined with flame ionization or nitrogen–phosphorus detection. With the latter method of detection, the limit of sensitivity was approximately 50 ng/mL; with the former, it was 125 ng/mL (Emonds *et al*., 1981).

Amsacrine has been measured in human nucleated haematopoietic cells by high-performance liquid chromatography (HPLC) after the leukocytes have been separated from the erythrocytes by dextran sedimentation (Brons *et al*., 1987). Amsacrine has also been determined in blood and urine by HPLC. The plasma samples were extracted with hexane at pH 3–4 and re-extracted with diethyl ether at pH 9 in the presence of borate present at a high concentration. Hexane extraction is not required for urine samples. After drying, the residue was dissolved in methanol before injection into the chromatograph. Absorbance was detected at 254 nm for plasma and simultaneously at 254 nm and 405 nm for urine samples (Paxton, 1984). Amsacrine has also been determined in serum by HPLC with a methanol:dichloromethane:acetate/diethylamine buffer (10:90:0.15) as eluent and ultraviolet detection at 254 nm. The limit of quantification with this method was 20 μg/L (Uges, 1990).

1.2 Production

Amsacrine is synthesized from 2′-methoxy-4′-nitrobutyranilide. The nitro group is reduced to the amine and converted to the methanesulfonamide, and the resulting free

amino group is reacted with 9-acridinyl chloride to yield amsacrine (Dubicki *et al.*, 1981; Gennaro, 1995).

Information available in 1999 indicated that amsacrine was manufactured and/or formulated in 18 countries (CIS Information Services, 1998; Swiss Pharmaceutical Society, 1999).

1.3 Use

Amsacrine is a cytotoxic drug used in the treatment of malignant disease. Its anti-tumour activity was first described in 1974 (Cain & Atwell, 1974), and the drug entered clinical trials in 1976 (Hornedo & Van Echo, 1985; Louie & Issell, 1985). It is an inhibitor of DNA topoisomerase II (Malonne & Atassi, 1997).

The use of amsacrine is limited almost exclusively to the treatment of leukaemia in adults and children, in which it has been included in a number of combination chemotherapy regimens at cumulative doses of 450–600 mg/m^2 (Arlin *et al.*, 1991; Berman, 1992). In phase II trials in patients with a variety of solid tumours, amsacrine showed little or no activity at typical doses of 90–150 mg/m^2, except in Hodgkin disease (Louie & Issell, 1985).

Amsacrine is formulated as two sterile liquids that are combined before intravenous administration, diluted in 500 mL dextrose and typically infused over 30–90 min (Editions du Vidal, 1998; Thomas, 1998).

1.4 Occurrence

Amsacrine is not known to occur as a natural product. No data were available to the Working Group on occupational exposure.

1.5 Regulations and guidelines

Amsacrine is not listed in any international pharmacopoeias. Information from an industry representative indicated that amsacrine is approved for use in at least 18 countries (Parke-Davis Canada, 1999).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Intraperitoneal administration

Mouse: In a bioassay for lung tumours, groups of 26 male and 26 female A/J mice, six to eight weeks of age, received seven weekly intraperitoneal injections of amsacrine [purity unspecified] in dimethyl sulfoxide and tricaprylin at a dose of 0 (vehicle control), 2, 5 or 10 mg/kg bw. Positive controls received a single intraperitoneal injection of 500 or 1000 mg/kg bw urethane. The mice were held for 17 weeks after the last injection. In the groups treated with amsacrine, no significant increase in the number of mice with lung adenomas was observed [tumour incidence and multiplicity not reported] (de la Iglesia *et al*., 1984).

3.2 Intravenous administration

Rat: Groups of 50 male and 50 female Wistar [Crl:(WI)BR] rats, six to eight weeks old, were given amsacrine (purity, 98.9%) intravenously into the lateral tail vein at a dose of 0 (vehicle control), 0.25, 1 or 3 mg/kg bw per day for five days, followed by a 23-day recovery period. This cycle of dosing and recovery was repeated six times. The animals were then maintained without dosing for the remainder of the 104-week study. The mortality rates were 44% of male controls, 48% at the low dose, 66% at the intermediate dose and 100% at the high dose; and 36% of female controls, 54% at the low dose, 46% at the intermediate dose and 96% at the high dose. None of the males at the high dose survived beyond week 90 of study. The incidences of small intestinal adenomas were 0/50, 0/50, 1/50 and 7/50 ($p < 0.01$, trend test) in male controls and those at the low, intermediate and high doses, and 0/50, 0/50, 0/50 and 7/50 ($p < 0.01$, trend test) in these groups of females, respectively. The incidences of small intestinal adenocarcinomas were 0/50, 1/50, 7/50 and 10/50 ($p < 0.01$, trend test) in the male groups and 0/50, 1/50, 1/50 and 9/50 ($p < 0.01$, trend test) in the female groups, respectively. Two adenocarcinomas and one adenoma of the large intestine were observed in males at the high dose and none in the other groups of males; two adenocarcinomas of the large intestine were observed in females at the high dose and none in the other groups. Squamous-cell carcinomas of the skin were observed in 1/50, 0/50, 4/50 and 10/50 ($p < 0.01$, trend test) rats in the four groups of males and in 0/50, 0/50, 0/50 and 4/50 ($p < 0.01$, trend test) rats in the four groups of females, respectively. Squamous-cell papillomas were observed at increased incidence in male rats (3/50 controls, 20/50 at the high dose; $p < 0.01$, Fisher's exact test) and in female rats (0/50 controls, 12/50 at the high dose; $p < 0.01$, Fisher's exact test). The incidences of keratocanthoma of the skin were significantly higher in male rats (3/50, 2/50, 7/50 and 12/50 in controls and at the low, intermediate and high doses, respectively; $p < 0.01$, trend test), but not in females. Fibromas of the skin occurred at significantly higher incidences in male rats (0/50, 8/50, 15/10 and 11/50; $p < 0.01$, trend test), but not in females. In females, the incidences of

mammary adenocarcinomas were 4/50, 2/50, 5/50 and 14/50 ($p < 0.01$, trend test) and the incidences of mammary fibroadenomas were 9/50, 9/50, 17/50 and 17/50 ($p < 0.01$, trend test) in the four groups, respectively (Gough *et al*., 1994; Graziano *et al*., 1996).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In cancer patients, amsacrine undergoes biphasic elimination, with a distribution half-time of 0.25–1.6 h (Van Echo *et al*., 1979; Jurlina *et al*., 1985; Linssen *et al*., 1993) and an elimination half-time of 4.7–9 h (Van Echo *et al*., 1979; Hall *et al*., 1983; Jurlina *et al*., 1985; Linssen *et al*., 1993). The total plasma clearance rate is 200–300 mL/min per m^2, and the apparent distribution volume is 70–110 L/m^2, suggesting concentration in tissues (Jurlina *et al*., 1985; Linssen *et al*., 1993). During a 1-h infusion of amsacrine at 90–200 mg/m^2, the peak plasma concentration was 10–15 μmol/L (Van Echo *et al*., 1979; Jurlina *et al*., 1985).

Although not fully reported, early trials in which amsacrine was given orally failed to reach the maximum tolerated dose, as shown by lack of toxicity even at doses as high as 500 mg/m^2 per day, suggesting incomplete or erratic absorption. In subsequent studies, the intravenous route was used, with which the maximum tolerated dose in patients with solid tumours is 100–150 mg/m^2 when administered over 1–3 h (described by Louie & Issell, 1985).

The elimination half-time was increased to 17 h in patients with impaired liver function, but it was not altered significantly in patients with renal impairment. Urinary excretion of amsacrine over 72 h, typically around 12% of the dose, decreased to only 2% in patients with renal impairment and increased to 20% in patients with hepatic impairment (Hall *et al*., 1983). After administration of [^{14}C]amsacrine, the total amount of radiolabel excreted in urine was 35% in patients with normal organ function, 49% in patients with liver impairment and 2–16% in patients with renal impairment. Patients with decreased amsacrine clearance rates experienced more toxicity. In two patients from whom biliary outflow was collected, 8% and 36% of the administered radiolabel was recovered within 72 h, < 2% being unchanged amsacrine (Hall *et al*., 1983).

Amsacrine is taken up rapidly by nucleated blood cells *in vivo*, peak concentrations occurring shortly after the end of a 3-h infusion; the concentration was about five times greater than the peak plasma concentration. Over 24 h, the mean integrated area under the time–concentration curve (AUC) for cellular amsacrine was eight times that of the AUC for plasma. The kinetics of elimination from peripheral blast cells was similar to that from plasma (Linssen *et al*., 1993).

In tumour samples from patients receiving amsacrine, the tumour:plasma concentration ratio ranged from 2:1 to 4.9:1 (Guo *et al*., 1983). High tissue concentrations of amsacrine were still present two weeks after treatment (Stewart *et al*., 1984), the highest concentrations occurring in the gall-bladder, liver and kidney. The concentrations in cerebrospinal fluid were < 2% of the corresponding plasma concentration in one study (Hall *et al*., 1983) and were undetectable in another (Guo *et al*., 1983).

About 97% of a dose of amsacrine is bound to protein bound in plasma in both cancer patients and healthy volunteers. Studies of human plasma *in vitro* showed no change in protein binding across a concentration range of 1–100 μmol/L. The unbound fraction increased to 21.7%, however, when the pH was changed to 6.4 (Paxton *et al*., 1986).

No studies of the metabolism of amsacrine in humans have been published.

4.1.2 *Experimental systems*

The pharmacokinetics of amsacrine has been described for mice (Cysyk *et al*., 1977; Kestell *et al*., 1990), rats (Cysyk *et al*., 1977), rabbits (Paxton & Jurlina, 1985) and dogs (Paxton *et al*., 1990). This typically includes biphasic elimination, with a rapid distribution phase and a more prolonged terminal elimination phase with a half-time of about 0.2 h in mice, 0.5 h in rats, 2.6 h in rabbits and 6.5 h in dogs. The pharmacokinetics was typically predictable in all species, including humans (Paxton *et al*., 1990).

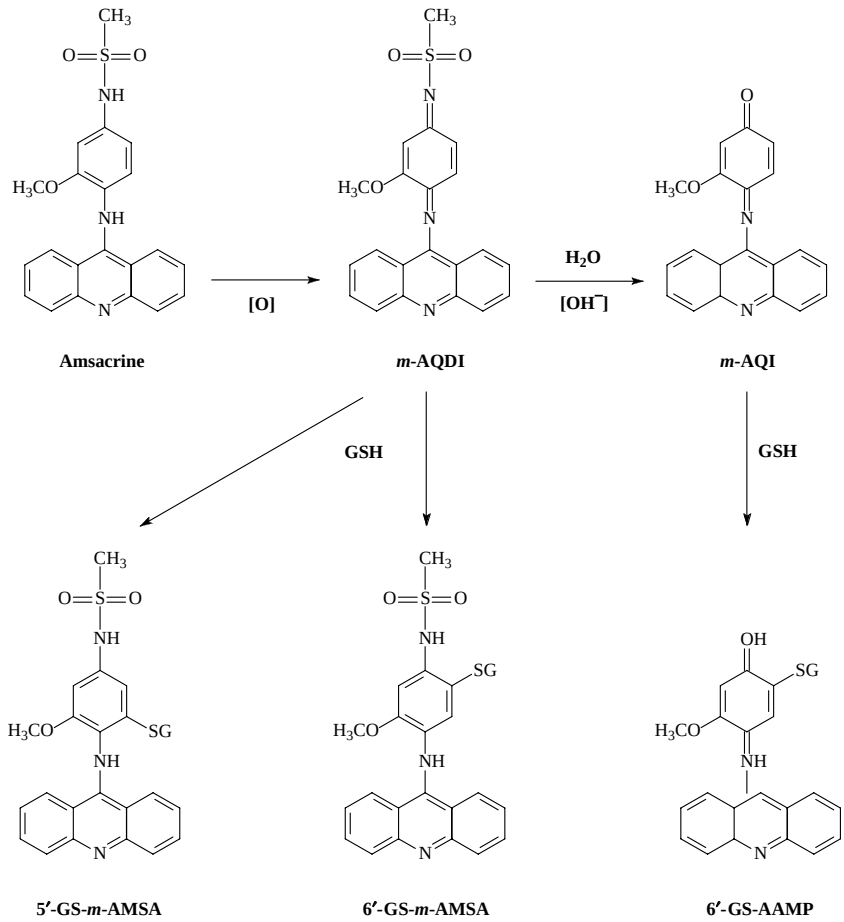
The bioavailability of orally administered amsacrine in mice (10 mg/kg bw) and rats (100 mg/kg bw) was incomplete and variable (Cysyk *et al*., 1978), with high concentrations occurring in the liver and rapid excretion into the bile. In rabbits, the bioavailability of amsacrine given orally at a dose of 12.7 μmol/kg bw was 50% in fed animals but 90% in fasting rabbits (Paxton, 1986).

After intravenous administration of [^{14}C]amsacrine to mice and rats, > 50% of the radiolabel was excreted in bile within the first 2 h, and the bile:plasma ratio was > 400:1 (Cysyk *et al*., 1977); 74% of an intravenous dose was excreted in the faeces of mice within 72 h (Robertson *et al*., 1988). These studies demonstrate the importance of the liver in clearance of amsacrine.

A number of reports have described the metabolism of amsacrine in rats and mice. In mouse bile, 5′- and 6′-glutathione conjugates were present in roughly equal amounts and accounted for 70% of the excreted biliary radiolabel after administration of radiolabelled amsacrine (Robertson *et al*., 1988). In rats, the principal biliary metabolite was the 5′-glutathione conjugate, which accounted for 80% of the excreted radiolabel within the first 90 min and > 50% of the administered dose over 3 h (Shoemaker *et al*., 1982). The 6′-conjugate was also subsequently identified in rat bile (Robertson *et al*., 1993). In rat liver microsomes and human neutrophils, intermediate oxidation products have been identified as *N*1′-methanesulfonyl-*N*4′-(9-acridinyl)-3′-methoxy-2′,5′-cyclohexadiene-1′,4′-diimine and 3′-methoxy-4′-(9-acridinylamino-2′,5′-cyclohexadien-1′-one (Shoemaker *et al*., 1984; Kettle *et al*., 1992). These oxidation products were about

100 times more cytotoxic to cells than amsacrine *in vitro*, while the principal conjugation product in rats, the 5′-glutathione conjugate, was inactive (Shoemaker *et al*., 1984). The same conjugation products were reported after exposure of Chinese hamster fibroblasts to amsacrine or its methanesulfonyl oxidation product in culture. The rate of glutathione conjugate formation during exposure to the oxidation product in cultured cells was rapid, whereas formation after exposure to amsacrine was slow, suggesting a low rate of oxidation of amsacrine to its oxidation products, with subsequent conjugation formation in this system (Robbie *et al*., 1990) (see Figure 1).

Figure 1. Metabolism of amsacrine



m-AQDI, N1′-methanesulfonyl-N4′-(9-acridinyl)-3′-methoxy-2′,5′-cyclohexadiene-1′,4′-diimine; *m*-AQI, 3′-methoxy-4′-(9-acridinylamino)-2′,5′-cyclohexadien-1′-one; 5′-GS-*m*-AMSA, 5′-glutathione conjugate of amsacrine; 6′-GS-*m*-AMSA, 6′-glutathione conjugate of amsacrine; 6′-GS-AAMP, 4′-(9-acridinylamino)-6′-(*S*-glutathionyl)-3′-methoxyphenol

4.2 Toxic effects

4.2.1 *Humans*

The toxicity of amsacrine in humans has been comprehensively reviewed and summarized (Hornedo & Van Echo, 1985; Louie & Issell, 1985). In all of the phase I studies, the dose-limiting toxic effect was myelosuppression, resulting mainly in leukopenia. Other effects included nausea, vomiting, fever, injection-site reaction, skin rash and discolouration (due to the yellow colour of the drug), mucositis and alopecia. Paraesthesia and hepatoxicity were seen in a few patients, but cardiac toxicity was not observed in one study (Louie & Issell, 1985).

In phase II studies with amsacrine as a single agent in patients with solid tumours, myelosuppression was again the dose-limiting effect (90–120 mg/m^2 once every three to four weeks), with leukopenia and thrombocytopenia occurring in almost all patients. Anaemia is also common. At these doses, the leukopenia is mild to moderate in most patients but more severe in around 30% of patients (Hornedo & Van Echo, 1985). The lowest counts usually occur at about day 10, with recovery by day 21. Myelosuppression is usually more severe in previously treated patients, and is much more severe with high doses of amsacrine (600–1000 mg/m^2).

Nausea, vomiting and mucositis are common after administration of amsacrine. Diarrhoea occurs in about 10–20% of patients (Louie & Issell, 1985). Stomatitis and mucositis become more frequent with higher doses (> 120 mg/m^2) (Slevin *et al.*, 1981).

Hepatoxicity has been reported, typically manifest as transient increases in serum bilirubin concentration and/or hepatic enzyme activity, but lethal hepatotoxicity has also been reported (Appelbaum & Shulman, 1982).

Phlebitis occurred in up to 17% of patients in early studies with amsacrine (Legha *et al.*, 1978; Louie & Issell, 1985), but the incidence has been reduced by administering the drug in a more dilute solution.

Eighty-two cases of amsacrine-associated cardiotoxicity were observed among over 6000 patients in phase II studies who had received amsacrine up until 1986, giving a total incidence of just over 1%. The more common effects were alterations in the electrocardiogram and arrhythmia, but cardiomyopathy and congestive heart failure also occurred (Weiss *et al.*, 1986). Amsacrine has been used safely in patients with preexisting arrhythmia when a serum potassium concentration of > 4 mmol/L was maintained (Arlin *et al.*, 1991).

4.2.2 *Experimental systems*

In dogs given single doses, the toxic effects at the highest non-lethal dose (3.1 mg/kg bw) were leukopenia, anaemia and increased serum activity of liver enzymes. Controls receiving 100 mg/mL *N*,*N*-dimethylacetamide and 2.8 mg/mL L-lactic acid (pH 3.2) also showed signs of liver toxicity. Similar toxic effects were seen at

the highest non-lethal regimes of five daily doses of 0.39 mg/kg bw in dogs and five daily doses of 5.2 mg/kg bw in rhesus monkeys (Henry *et al.*, 1980).

Toxic effects on the gastrointestinal and central nervous system were observed at lethal doses in dogs (6.25 mg/kg bw as a single dose, 0.78 mg/kg with five daily doses), but no cardiac toxic effects were reported in any species (Henry *et al*., 1980). In subsequent studies, evidence of cardiotoxicity was not seen in rats (Kim *et al*., 1985), but cardiac rhythm abnormalities and ectopic pulses were seen in rabbits at doses of 2.5–7.5 mg/kg bw and in an isolated rabbit heart preparation, in which dose-related negative ionotropic effects were seen at therapeutically relevant concentrations (D'Alessandro *et al*., 1983).

Intravenous dosing of rats at 1 or 3 mg/kg bw per day for five days resulted in hair loss, diarrhoea and leukopenia; these effects were reversible (Pegg *et al*., 1996).

Local tissue reactions were seen when the drug was administered subcutaneously or intramuscularly to guinea-pigs or rabbits, but similar effects were seen after administration of the vehicle alone, suggesting that the acidity of the vehicle (see above) may have been responsible (Henry *et al*., 1980). Skin rashes in personnel involved in bulk formulation of amsacrine prompted further studies in experimental animals. In the Magnussen and Kligman maximization test, amsacrine was extremely sensitizing to the skin of guinea-pigs when given as a challenge dose by direct application, while the vehicle alone produced almost no response. The animals were not sensitized for systemic anaphylaxis, however, and there was no detectable induction of antibodies in rabbits (Watson *et al*., 1981).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Amsacrine at a dose of 40 mg/m^2 per day for three days every three weeks led to a marked reduction in sperm count in a patient with melanoma (da Cunha *et al*., 1982). As in mice (da Cunha *et al*., 1985), however, the sperm count returned to normal during a 10-week gap in treatment, indicating that amsacrine has only a temporary, reversible effect on differentiating germinal cells and is not toxic to stem cells.

4.3.2 *Experimental systems*

In mice given total doses of 7.5–30 mg/kg bw amsacrine intraperitoneally as three daily doses or as a single dose of 15 mg/kg bw, substantial killing of differentiating spermatogonia (types A_2 to B) was seen. There was no effect on post-spermatogonial stages and little effect on stem cells, and the sperm counts had recovered by day 56 (da Cunha *et al*., 1985).

Amsacrine was reported in an abstract to be embryotoxic, fetotoxic and teratogenic in groups of 20 CD rats dosed intraperitoneally with 0.1, 0.5 or 1.0 mg/kg bw per day amsacrine lactate on days 6–15 of gestation. Maternal weight gain was reduced at the

high dose only. Eye, jaw and other skeletal malformations were observed in the fetuses at all doses. An increased frequency of resorptions and decreased fetal weight were observed at the intermediate and high doses (Ng *et al*. 1987).

Day-10 rat embryos [strain not specified] cultured for 24 h *in vitro* were exposed for the first 3 h to amsacrine at concentrations of 10 nmol/L to 1 μmol/L. A dose-related increase in the frequency of malformations was observed at doses of 50–500 nmol/L, and 100% of the embryos were malformed at 500 nmol/L. At 1 μmol/L, all embryos were killed. Embryonic growth was reduced at concentrations from 200 nmol/L. The malformations consisted mainly of hypoplasia of the prosencephalon, microphthalmia and oedema of the rhombencephalon. Similar malformations were observed in the same system with etoposide (see the monograph on etoposide). Comparison of the concentrations necessary to produce lethality and malformations in 50% of fetuses showed that amsacrine was 10 times and 20 times more potent, respectively, than etoposide (Mirkes & Zwelling, 1990).

In a study reported only as an abstract, male mice were treated with a maximum tolerated dose of 15 mg/kg bw [no further details given] amsacrine and showed no signs of dominant lethal mutation. Female mice treated with a maximum tolerated dose of 12.5 mg/kg bw amsacrine in a test for total reproductive capacity showed reduced litter size at the first mating interval, suggesting a dominant lethal effect (Bishop *et al*., 1997).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

General reviews on the mutagenicity of inhibitors of DNA topoisomerase II enzymes, including amsacrine, have been published (Anderson & Berger, 1994; Ferguson & Baguley, 1994, 1996; Baguley & Ferguson, 1998). Jackson *et al*. (1996) collated a genetic activity profile for this drug. The results are summarized in Table 1.

Amsacrine was mutagenic in some strains of *Salmonella typhimurium*, causing an increased number of revertants in TA1537, a small increase (about twofold) in revertants in TA102 but no increase in revertants in TA1535, TA98 or TA100. Addition of an exogenous metabolic system reduced but did not eliminate the mutagenic effects in TA1537. The positive effects required a dose of about 800 μg/plate, which is higher than those tested in mammalian cells.

Amsacrine reverted a frameshift mutant of T4 and induced prophage λ in *Escherichia coli* WP2, suggesting an 'SOS' repair response. In *Saccharomyces cerevisiae* strain D5, amsacrine failed to induce the mitochondrial 'petite' mutation, but it was an effective mitotic recombinogen when testing was done under conditions permitting cell growth. It did not induce either forward or reverse mutations in *Neurospora crassa.*

Table 1. Genetic and related effects of amsacrine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli WP2s (λ) prophage induction, SOS response	–	+	312	DeMarini & Lawrence (1992)
Bacteriophage T4, reverse mutation (frameshift)	+	NT	1	DeMarini & Lawrence (1988)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	1000 μg/plate	Ferguson *et al.* (1988)
Salmonella typhimurium TA102, reverse mutation	(+)	(+)	800 μg/plate	Ferguson *et al.* (1988)
Salmonella typhimurium TA102, reverse mutation	–	NT	3.33 μg/plate	Albertini *et al.* (1995)
Salmonella typhimurium TA1535, reverse mutation	–	NT	50 μg/plate	Iwamoto *et al.* (1992a)
Salmonella typhimurium TA1537, reverse mutation	+	+	100 μg/plate	Ferguson *et al.* (1988)
Salmonella typhimurium TA1537, reverse mutation	+	NT	225 μg/plate	Iwamoto *et al.* (1992a,b)
Saccharomyces cerevisiae D5, mitotic recombination	+	NT	500	Ferguson & Turner (1988a)
Saccharomyces cerevisiae D5, mitochondrial petite mutation	–	NT	> 2000	Ferguson & Turner (1988b)
Neurospora crassa, forward mutation, *ad-3A* frameshift strain	–	NT	100	Gupta (1990)
Neurospora crassa reverse mutation, *ad-3A* frameshift strain	–	NT	315 μg/plate	Gupta (1990)
Drosophila melanogaster, genetic crossing-over or recombination (*white–ivory* assay)	–		790 in feed	Ferreiro *et al.* (1997)
Drosophila melanogaster, somatic mutation and recombination	–		1970	Torres *et al.* (1998)
DNA single-strand breaks and DNA–protein cross-links, mouse L1210 cells *in vitro*	+	NT	0.04	Minford *et al.* (1984)
DNA single- and double-strand breaks, Chinese hamster lung V79 cells *in vitro*	+	NT	0.04	Pommier *et al.* (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks (protein-linked), mouse embryo 3T3 fibroblasts *in vitro*	+	NT	0.2	Markovits *et al.* (1987)
DNA single-strand breaks, mouse leukaemia L1210 cells *in vitro*	+	NT	4.0	Covey *et al*. (1988)
DNA–protein cross-links, mouse leukaemia L1210 cells *in vitro*	+	NT	0.4	Covey *et al*. (1988)
DNA double-strand breaks, mouse fibrosarcoma 935.1 cells *in vitro*	+	NT	NR	Woynarowski *et al*. (1994)
DNA–protein cross-links and double-strand breaks, Chinese hamster ovary CHO-K1 and *xrs-1* cells *in vitro*	+	NT	0.2	Caldecott *et al*. (1990)
Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	NT	0.2	Wilson *et al*. (1984)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus *in vitro*	+	NT	0.004–0.04	Wilson *et al*. (1984)
Mutation, Chinese hamster lung V79 cells, *Hprt* locus *in vitro*	+	NT	0.08	Pommier *et al*. (1985)
Gene mutation, Chinese hamster ovary AA8 cells, *Hprt* locus *in vitro*	+	NT	NR	Ferguson *et al*. (1992)
Mutation, Chinese hamster ovary AA8 cells, cytosine arabinoside resistance *in vitro*	(+)	NT	NR	Ferguson *et al*. (1992)
Mutation, A_L (human × hamster) hybrid cell line, *Hprt* locus *in vitro*	(+)	NT	0.016	Shibuya *et al*. (1994)
Mutation, A_L (human × hamster) hybrid cell line, S1 phenotype *in vitro*	+	NT	0.004	Shibuya *et al*. (1994)
Mutation, Chinese hamster ovary D422 cells, *Aprt* locus *in vitro*	+	NT	0.4	Zhou *et al.* (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	0.001	DeMarini *et al*. (1987)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	0.001	Doerr *et al*. (1989)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	0.001	Backer *et al*. (1990)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	NT	0.05	Albertini *et al.* (1995)
Gene mutation, mouse lymphoma L5178Y cells, *Hprt* locus *in vitro*	+	NT	0.001	Albertini *et al.* (1995)
Mutation, AS52 Chinese hamster cells, bacterial *Gpt* locus *in vitro*	+	NT	0.04	Ferguson *et al*. (1998)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	0.4	Lim *et al*. (1986)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	NT	0.08	Pommier *et al*. (1985)
Sister chromatid exchange, Chinese hamster lung DC3F cells *in vitro*	+[c]	NT	0.4	Pommier *et al.* (1988)
Sister chromatid exchange, Chinese hamster AA8 and EM9 cells *in vitro*	+	NT	0.4	Cortés *et al*. (1993)
Sister chromatid exchange, Chinese hamster CHO6 cells *in vitro*	+	NT	0.1	Cortés & Piñero (1994)
Sister chromatid exchange, Chinese hamster CHO6 cells *in vitro*	+	NT	0.04	Piñero *et al*. (1996)
Micronucleus formation, mouse C3H10T1/2 cells *in vitro*	+	NT	0.005	Ferguson *et al.* (1986)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.001	Doerr *et al*. (1989)
Micronucleus formation, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.001	Backer *et al*. (1990)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations (anaphase/telophase test), Chinese hamster ovary cells *in vitro*	+	NT	0.1	Larripa *et al.* (1984)
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	0.005	Ferguson *et al.* (1988)
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	0.08	Pommier *et al.* (1985)
Chromosomal aberrations, Chinese hamster lung DC3F cells *in vitro*	+[c]	NT	0.4	Pommier *et al.* (1988)
Chromosomal aberrations, Chinese hamster AA8 and EM9 cells *in vitro*	+	NT	0.4	Cortés *et al.* (1993)
Chromosomal aberrations, Chinese hamster CHO6 cells *in vitro*	+	NT	0.1	Cortés & Piñero (1994)
Chromosomal aberrations, mouse L1210 cells *in vitro*	+	NT	0.04	Ferguson & Baguley (1984)
Chromosomal aberrations, C3H10T1/2 mouse cells *in vitro*	+	NT	0.0025	Ferguson *et al.* (1986)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.001	Doerr *et al.* (1989)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.0005	DeMarini *et al.* (1987)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.0001	Backer *et al.* (1990)
Aneuploidy/polyploidy, Chinese hamster–human hybrid GM10115A cell line *in vitro*	+	NT	0.004	Ferguson *et al.* (1996a)
Polyploidy, Chinese hamster ovary cells *in vitro*	+	NT	16	Sumner (1995)
Polyploidy, murine erythroleukaemic cells *in vitro*	+	NT	1	Zucker *et al.* (1991)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, C3H10T1/2 mouse cells	+	NT	0.01	Ferguson *et al*. (1986)
Protein-associated DNA strand breaks, human leukaemic myeloblasts and normal lymphocytes *in vitro*	+	NT	0.1	Brox *et al*. (1986)
DNA double-strand breaks, human breast cancer T-47D cells *in vitro*	+	NT	0.15	Epstein & Smith (1988)
DNA double-strand breaks, human breast cancer MCF-7 cells *in vitro*	+	NT	0.2	Bunch *et al.* (1994)
DNA single- and double-strand breaks, Raji (Burkitt lymphoma) cells *in vitro*	+	NT	1.2	Johnson & Beerman (1994)
DNA double-strand breaks within the *AML*1 locus, various human leukaemia cell lines *in vitro*	+	NT	12	Stanulla *et al*. (1997)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.005	Kao-Shan *et al*. (1984)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.2	Andersson & Kihlman (1989)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	3.2	Ribas *et al*. (1996)
Micronucleus formation, human lymphocytes from neonatal cord blood *in vitro*	+	NT	0.01	Slavotinek *et al*. (1993)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.005	Kao-Shan *et al*. (1984)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.6	Andersson & Kihlman (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.4	Mosesso *et al*. (1998)
Chromosomal aberrations, Hela cells *in vitro*	+	NT	0.25	Ferguson & Baguley (1984)
DNA breaks preferentially in episomal regulatory regions in tumour-bearing mRIII S/J mice *in vivo*	+		15 iv × 1	Cullinan *et al*. (1990)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, host-mediated assay, L1210 leukaemia cells grown intraperitoneally in male DBA/2J mice	+		5 ip × 1	Ferguson & Baguley (1984)
Sister chromatid exchange, bone-marrow cells of male C57BL/6J mice *in vivo*	+		1.5 ip × 1	Backer *et al*. (1990)
Micronucleus formation, bone-marrow cells of male and female CFW mice *in vivo*	+		1.5 ip × 1	Larripa *et al*. (1984)
Micronucleus formation, bone marrow of male and female CD-1 mice *in vivo*	+		1.5 ip × 1	Holmström & Winters (1992)
Micronucleus formation, hepatocytes and peripheral blood reticulocytes of male ddY mice *in vivo*	+		10 ip × 1	Igarashi & Shimada (1997)
Chromosomal aberrations, bone marrow of male C57BL/6J mice *in vivo*	+		3 ip × 1	Backer *et al*. (1990)
Dominant lethal mutations, female C3H × C57BL mice	+		12.5[d]	Bishop *et al*. (1996)
Dominant lethal mutations, male C3H × C57BL mice	–		15[d]	Bishop *et al*. (1996)
Sister chromatid exchange, human lymphocytes *in vivo*	–		30 mg/m^2 per day; continuous iv, 3–4 d	Kao-Shan *et al*. (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vivo*	+		30 mg/m^2 per day; continuous iv, 3–4 d	Kao-Shan *et al.* (1984)

[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 per day; NR, not reported; ip, intraperitoneally; iv, intravenously; d, day
[c] Negative in topoisomerase II-resistant DC3F/9-OHE cells
[d] Route not specified

Amsacrine is a clastogen in mammalian cells. It caused DNA double-strand breaks and DNA–protein cross-links in various animal cells and in human cell lines at a concentration of about 1 μmol/L. Amsacrine caused DNA breaks preferentially in episomal regulatory regions in tumour-bearing mRIII S/J mice and at a very specific site within the *AML*1 locus in several human cell lines. The Chinese hamster cell line xrs-1 was hypersensitive to amsacrine treatment (Caldecott *et al.*, 1990). The drug appears to inactivate DNA synthesis by inhibiting replicon cluster initiation (Suciu, 1991).

Amsacrine caused chromosomal aberrations in cultured Chinese hamster cells, in various rodent cell lines, in HeLa cells and in cultured human peripheral blood lymphocytes. It also induced micronuclei in neonatal human lymphocytes. Fluorescence in-situ hybridization techniques revealed a high frequency of dicentrics and stable translocations in amsacrine-treated human peripheral blood lymphocytes. Various chromosomal aberrations were also observed in mouse leukaemia L1210 cells that were grown intraperitoneally in male DBA/2J mice and treated with amsacrine at doses and schedules that effectively reduced the tumour burden. Additionally, amsacrine induced micronuclei and chromosomal aberrations in the bone marrow of non-tumour-bearing male and female mice. In male ddY mice, amsacrine increased the incidence of micronuclei in both hepatocytes and peripheral blood reticulocytes. In one study, amsacrine caused chromosomal aberrations, but no sister chromatid exchange in blood lymphocytes of patients treated with this drug by intravenous infusion.

Amsacrine induced sister chromatid exchange in Chinese hamster cells and in human lymphocytes *in vitro*. This effect was also seen *in vivo*. It had no effect in *Drosophila melanogaster* in the wing spot test or in the *white–ivory* assay, which provide a measure of somatic crossing-over or recombination.

Although there is evidence that amsacrine causes point mutations in bacteria, it does not appear to do so in mammalian cells, possibly because the concentrations necessary to evoke these events would be lethal to mammalian cells. Amsacrine did not induce resistance to ouabain (a known measure of base-pair substitution mutagenesis) in Chinese hamster lung V79 cells, although it induced mutations at the *Hprt* locus in these cells, in Chinese hamster ovary cells and in A_L (human × hamster) hybrid cell lines. It was a potent mutagen at the *MIC*1 locus in the last cell line and a moderate inducer of cytarabine resistance (by an unknown mechanism) in Chinese hamster AA8 cells. Amsacrine caused mutations at the *Aprt* locus in Chinese hamster ovary D422 cells. In two of three studies, it induced primarily small colony mutants at the *Tk* locus in mouse lymphoma L5178Y cells; although these events were classified as gene mutations (Jackson *et al.*, 1996), they are probably chromosomal events. Mutations at the *Hprt* locus in V79 cells paralleled chromosomal events as measured by micronucleus formation (Wilson *et al.*, 1984), strand breakage (single or double strands) and sister chromatid exchange. The differential sensitivity of the *MIC*1 locus (studied with the S1 antigen) and the *Hprt* locus in A_L cells suggests that amsacrine produces megabase-pair deletions at the *Hprt* locus that would prove lethal. Amsacrine primarily increased small colony mutants in mouse lymphoma L5178Y

cells, and these are known to be caused by chromosomal mutations (DeMarini *et al*., 1987).

The molecular nature of amsacrine-induced mutations to 6-thioguanine resistance was studied in late log-phase Chinese hamster AS52 cells (Ferguson *et al*., 1998). Neither frameshift nor base pair-substitution mutational events could be unequivocally associated with this treatment. In the study of Ferguson *et al*. (1998), amsacrine caused major chromosomal deletions and illegitimate recombination in Chinese hamster ovary cells, detectable by Southern blotting. On the basis of the size of the deletions observed, Shibuya *et al*. (1994) speculated that amsacrine-induced deletions are mediated by a series of subunit exchanges between overlapping DNA topoisomerase II dimers at the bases of replicons or larger chromosomal structures such as replicon clusters or chromosomal minibands. Zhou *et al*. (1997) also showed reciprocal exchanges involving the *Aprt* locus after amsacrine treatment and suggested a model similar to that of Shibuya *et al*. (1994).

The extent of amsacrine-induced mutation varies among cell lines, depending on their susceptibility to apoptosis, or programmed cell death, which is a means of ensuring that genetically damaged cells do not survive to form progeny and acts as an alternative pathway to mutagenesis. For example, treatment of human lymphoblastoid AHH-1 $TK^{+/-}$ cells with amsacrine led to cell cycle arrest at the G_2/M phase, and conditions that enhance apoptosis led to a low recovery of viable mutants (Morris *et al*., 1995, 1996). Cells may be particularly susceptible to apoptosis by DNA topoisomerase II inhibitors such as amsacrine, which cause apoptosis through both *p53*-dependent and -independent routes (Ferguson, 1998).

Fluorescence in-situ hybridization techniques revealed that amsacrine caused both aneuploidy and polyploidy in a Chinese hamster–human cell hybrid. Polyploidy was also demonstrated by cytogenetic techniques in Chinese hamster ovary cells and, by flow cytometry, in murine erythroleukaemic cells. Amsacrine caused cell transformation in mouse C3H10T1/2 cells *in vitro* and prevented the dimethyl sulfoxide-induced differentiation of human leukaemia HL-60 cells.

Amsacrine also mutates germ cells: dominant lethal events were seen in female but not in male mice. Treatment of meiotic cells with amsacrine can disrupt the structure of the synaptonemal complex, a meiosis-specific structure that is essential for accurate recombination and chromosomal segregation. For example, exposure of preleptotene mouse germ cells to amsacrine led to an aberrant multi-axial configuration of the synaptonemal complex (Ferguson *et al*., 1996b). This provides indirect evidence that amsacrine interferes with meiotic recombination and is a probable aneuploidogen in meiotic cells.

4.5 Mechanistic considerations

In general, the events caused by amsacrine in mammalian cells *in vitro* occurred in the absence of exogenous metabolic activation, although a possible mechanism for

oxidative metabolism has been identified (Kettle *et al*., 1992). Three mechanisms have been identified to explain the mutagenicity and carcinogenicity of amsacrine.

It has three activities that may be responsible for mutation.

1. It inhibits DNA topoisomerase II enzymes: Amsacrine is a DNA topoisomerase II poison that has been shown to promote DNA cleavage, with a strong preference for a site one base away from adenine (Marsh *et al.*, 1996). Most of the mutational events reported in mammalian cells, including point mutations, chromosomal deletions and exchanges and aneuploidy, can be explained by this activity. Amsacrine does not inhibit bacterial topoisomerases and may not mutate bacterial cells by the same mechanism as mammalian cells.

2. It possesses readily oxidizable functions: The anilino ring of amsacrine can be reversibly oxidized, either chemically or microsomally, to produce a quinone diimine (Jurlina *et al.*, 1987). DeMarini and Lawrence (1992) suggested that the induction of prophage reflects this activity of the drug. Nevertheless, none of the mutations seen with amsacrine is of the type usually associated with reactive oxygen species.

3. It intercalates into, but does not interact covalently with, DNA: DNA intercalation, but not DNA topoisomerase II inhibition, is probably responsible for the frameshift mutagenicity seen in bacteria (Ferguson & Baguley, 1981; Ferguson & Denny, 1990).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Amsacrine is a synthetic DNA topoisomerase II inhibitor used primarily in the treatment of leukaemia in adults and children.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Amsacrine was tested by intraperitoneal administration in one assay for lung adenomas in mice; no increase in incidence was reported. In a single study in rats given amsacrine by intravenous administration, small-intestinal adenomas and adenocarcinomas were induced in a dose-dependent fashion in males and females, and a few adenocarcinomas of the large intestine were seen in males and females at the high dose. The incidences of squamous-cell papillomas and carcinomas of the skin were increased in males and females, those of keratoacanthomas and of fibromas of the skin were

increased in males, and those of mammary fibroadenomas and adenocarcinomas were increased in females. All of these increases were dose-dependent.

The occurrence of intestinal carcinomas in rats of each sex and the occurrence of skin tumours after intravenous administration of a chemical are unusual.

5.4 Other relevant data

In humans, amsacrine is eliminated biphasically, with an elimination half-time of 5–9 h. The drug is rapidly taken up by nucleated blood cells, with an overall cell:plasma ratio over 24 h of 8:1, and is distributed to other tissues. Preliminary studies suggest that the oral bioavailability of amsacrine is poor, and there is currently no oral formulation of the drug. About 35% of an intravenous dose was excreted renally over 72 h, with 12% as unchanged amsacrine; biliary recovery in two patients was up to 36%. Biphasic elimination was also observed in a number of animal species. In mice and rats, > 50% of a radiolabelled dose was excreted in the bile within 2 h, and 74% of the dose was recovered in the faeces of mice by 72 h. The results of studies in humans and animals demonstrate the importance of renal and hepatic function in amsacrine clearance. In animals, much of a radiolabelled dose of amsacrine was excreted as metabolites, some of which were cytotoxic. There are currently no data on the metabolism of amsacrine in humans.

In human and animal species, the main toxic effect of amsacrine is myelosuppression, especially leukopenia. Other common toxic effects are nausea and vomiting, mucositis, alopecia and diarrhoea. Less common effects include hepatotoxicity and cardiotoxicity.

Amsacrine does not bind covalently to DNA. It appears to mutate cells through two mechanisms. In mammals, amsacrine is an effective poison of DNA topoisomerase II enzymes, leading to cellular accumulation of protein-masked double-stranded DNA breaks and, with time, a variety of chromosomal aberrations. It is also an effective recombinogen, its predominant effects appearing to involve the deletion and/or interchange of large DNA segments. Amsacrine also induces both polyploidy and aneuploidy. It is a frameshift mutagen in bacteria and bacteriophages, and this property may be related to its intercalating action.

Potent protein-masked DNA breakage and clastogenic effects occur in human cells *in vitro* and in animal cells *in vivo*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of amsacrine.

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

Overall evaluation

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

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