Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of daminozide in the context of Council Directive 91/414/EEC[1]

(Question N° EFSA-Q-2003-120)

adopted on 11 May 2004

SUMMARY OF OPINION

The existing active substance daminozide is currently under review for inclusion in Annex I to Council Directive 91/414/EEC. The supported use for daminozide is as a growth regulator on ornamentals under glass. The issue still to be resolved relates to the metabolite UDMH and the possible effects on operators applying the substance or workers after re-entry into greenhouses where ornamental plants have been treated with daminozide.

The RMS concluded that, although it cannot be excluded that UDMH is genotoxic and carcinogenic, the use of daminozide and hence exposure to UDMH poses a very low risk to operators and workers. Since these arguments were not accepted by all Member States, Health and Consumer Protection Directorate-General from the European Commission requested the comments of the Scientific Panel on Plant health, Plant protection products and their Residues (PPR) on the following question: "*The Scientific Panel on Plant health, Plant protection products and their Residues is requested to comment on the mechanism of action of the carcinogenic response of rodents to UDMH and to indicate whether or not a threshold could be derived for this effect. If yes, the PPR Panel is requested to indicate this value".*

The Scientific Panel on Plant health, Plant protection products and their Residues, having regard to the submitted question, concludes that, on the basis of the available data, it is not possible to identify the mechanism responsible for the carcinogenic action of UDMH in rodents. There is no *in vitro* evidence of genotoxicity of pure and oxidation-protected UDMH and *in vivo* studies are not available. In addition, the PPR Panel noted an apparent discrepancy in that long-term studies with daminozide did not give rise to carcinogenicity in rats and mice at doses that should have produced internal doses of metabolically-formed UDMH at least one order of magnitude higher than those proven to be carcinogenic on direct testing. In addition, N7-methylation of guanine was found to be 50-fold higher in a study following oral administration of UDMH to rats, when compared to the corresponding data for daminozide. Thus, the PPR Panel considered that any conclusion on the mechanism of carcinogenicity of orally administered UDMH should be regarded as including a degree of uncertainty. The PPR Panel concluded that the weight of evidence is against a genotoxic mechanism.

Among possible non-genotoxic mechanisms, altered regulation of cell proliferation or hormonal imbalance are plausible alternatives to genotoxicity, but these mechanisms have not been specifically investigated and thus a more definitive conclusion on the mechanism involved is not possible at the moment.

In experimental testing of UDMH for carcinogenicity in rats and mice, no effects were observed at 0.09 mg/kg bw/day and 1.41 mg/kg bw/day, respectively.

If the observed UDMH carcinogenicity is due to a non-genotoxic mechanism, the above indicated doses should be considered to be toxicological thresholds. However, taking together the uncertainties associated with the mechanism and the possibility that UDMH in greenhouse conditions may form oxidised derivatives that might be genotoxic, the PPR Panel is of the opinion that any use of these doses as thresholds should be undertaken only with due caution.

[1] For citation purposes: Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of daminozide in the context of Council Directive 91/414/EEC, *The EFSA Journal* (2004), 61, 1-27.

Key words : daminozide, growth regulator, ornamentals, toxicology, genotoxicity, carcinogenicity.

TABLE OF CONTENTS

BACKGROUND[2]

Daminozide is used as a plant growth regulator and is included in the first list of active substances referred to in Article 8(2) of Directive 91/414/EEC[3] concerning the placing of plant protection products on the market. On the basis of the evaluation report prepared by the

[2] The Background and the Terms of reference were sent by the Commission.

[3] OJ No L 230, 19.08.1991, p.1.

Netherlands as Rapporteur Member State (RMS), the substance has been peer reviewed with Member State experts and consequently discussed in the working group "Plant Protection Products – Evaluation and Legislation" of the Standing Committee on the Food Chain and Animal Health.
The peer review identified several data gaps which were addressed by the notifier. All information submitted has been evaluated and discussed with Member States in the Working Groups "Evaluation" and "Legislation". The supported use(s) for daminozide is (are) as a growth regulator on ornamentals under glass. The minimum purity of daminozide as manufactured is 990 g/kg with maximum 30 mg/kg 1,1-dimethylhydrazide (UDMH) and 2 mg/kg N-nitrosodimethylamine as impurities (NDMA).

The remaining issue to be resolved relates to the metabolites NDMA/UDMH and their possible effects on operators applying daminozide or workers after re-entry into greenhouses where ornamentals have been treated with daminozide.
NDMA could not be detected in air nor as dislodgeable foliar residue after application.
UDMH on the contrary was detected at low levels both in air and as dislodgeable residues.
The Rapporteur Member State the Netherlands concluded that the submitted information does not allow exclusion of the possibility that UDMH has *in vivo* genotoxic and carcinogenic effects.
Health and Consumer Protection Directorate-General from the European Commission requested on 22 December 2003 the comments of the Scientific Panel on Plant health, Plant protection products and their Residues (PPR) on the following question.

TERMS OF REFERENCE

The Scientific Panel on Plant health, Plant protection products and their Residues is requested to comment on the mechanism of action of the carcinogenic response of rodents to UDMH and to indicate whether or not a threshold could be derived for this effect. If yes, the PPR Panel is requested to indicate this value.

ASSESSMENT

1.1. Introduction

The PPR Panel, after an extensive search in the literature noted that, in addition to the documentation provided to EFSA, there is a large body of published papers dealing with UDMH and, in addition, reviews on UDMH toxicity were performed by JMPR[4] in 1991, US-EPA[5] in 1993 IARC[6] and in 1999 . All these papers were considered in the preparation of this opinion.
Given the nature of the question submitted to the PPR Panel, relevant aspects to be discussed in the formulation of this opinion include:

- Metabolic and kinetics studies on daminozide/UDMH
- Mutagenicity studies *in vitro* and *in vivo* of UDMH
- Carcinogenicity studies of daminozide
- Carcinogenicity studies of UDMH

[4] Joint Meeting on Pesticide Residues
[5] US-EPA: USA Environmental Protection Agency, the R.E.D. facts - September 1993
[6] IARC: International Agency Research Cancer

1.2. Metabolic and kinetic studies on daminozide/UDMH

An oral metabolism study of radiolabeled daminozide in male rats was conducted by Slauter *et al.* (1993). Radioactivity was detected in urine, feces, and expired CO_2. Recovery of the administered dose from all sources was 89%. Daminozide was the predominant radiolabeled compound in the urine at 6 hr, but by 24 hr, its metabolite, UDMH, was the predominant radiolabeled compound in the urine. In the first 48 hours following dosing, about 29% of the dose was excreted in the urine as UDMH and 13% as daminozide. Based on the excretion data, at least 57% of an oral dose of daminozide was absorbed into the systemic circulation with at least 47% excreted in the urine, 32% in the feces and 7% as $^{14}CO_2$. The terminal body burden averaged 2% of the administered dose. At the 96 hr sacrifice, the highest tissue levels of radioactive compounds were in liver and blood.

It has to be noted that this study was carried out with a single, low dose of daminozide (1 mg/kg bw) while another study is available in male rats at a single, higher dose (45 mg/kg bw) (Fathulla, 1999). This latter study showed evidence of initial saturation of the absorption process and therefore the overall best estimate of the extent of oral absorption of daminozide was considered by the RMS to be about 35%. This has implications on the estimated amount of UDMH formed in the organism after high dose daminozide intake: it is probable that the saturation of the absorption process limits the percentage of the dose that can be converted into UDMH in male rats to below 29%.

UDMH was also present in urine in substantial amounts (but lower than daminozide) after 96 hr in another metabolism study in miniature swine (Mitros, 1987).

No other studies are available on daminozide metabolite formation and distribution; in particular no studies are available in the species and sex of interest on the basis of the carcinogenicity results (i.e. female rats and mice).

No metabolic studies are available on UDMH administered to either rats or mice.

The PPR Panel noted that in the male rat a substantial amount of administered daminozide (about 29% at low dose and less at high dose) was metabolically converted into UDMH and recovered as such in the urine. Since in the male rat the dietary administration of up to 10,000 ppm daminozide was not found to cause carcinogenic effects, it may be inferred that metabolically-formed UDMH, up to a dose representing a substantial proportion of the administered dose of daminozide, was not effective in causing carcinogenic effects in this species and sex.

1.3 Mutagenicity studies *in vitro* and *in vivo* of UDMH

Apart from the studies submitted by the notifiers[7], a large body of mutagenicity studies on UDMH have been published in the open literature. The full list of available studies is reported in the Appendix - Table 1.

When assessing the results of genotoxicity tests of UDMH, it has to be considered that this chemical after exposure to light and air may generate oxidised derivatives, among which is dimethylnitrosamine (NDMA), a substance showing genotoxic properties after metabolism. To avoid the formation of such products, UDMH has to be protected against oxidation during its handling, in experimental studies.

The interpretation of the results of many of the tests reported in Table 1 of the Appendix is made uncertain by the lack of specification of the purity of the tested compound and by the lack of indication of whether or not UDMH handling was performed with the necessary precautions to avoid its oxidation. In Table 1, the few studies undertaken with "protected" UDMH are identified and marked with an asterisk. The interpretation of positive studies with regard to the genotoxic potential of UDMH should be made with caution whenever this confounder cannot be excluded.

1.3.1 *In vitro* assays

A summary evaluation of the available studies is as follows:

- ***MUTATIONS AT THE GENE LEVEL IN PROKARYOTES***

Regarding the bacterial reverse mutation assay based on the reversion rate, there is a large number of studies in the *Salmonella typhimurium* TA100, TA1535, TA1537 and TA1538 tester strains in the absence or the presence of metabolic activation systems (S9 mix). The large majority of these studies were negative. Among the studies reported to be positive, a study on TA100 and TA102 (the only test available with this strain) was performed with UDMH oxalate at unknown concentrations (Matsushita *et al.*, 1993); the positive study by Bruce and Heddle (1979), that also tested in TA98, used a test material of doubtful purity as it provided an unusually high LD50.

Weakly positive results were found in the *Salmonella typhimurium* TA98 tester strain in the absence or the presence of metabolic activation systems (S9 mix) (De Flora, 1981; Parodi *et al.*, 1981). The PPR Panel, however, noted that it is surprising to find positive results occurring

[7] Fine Agrochemicals Ltd and Crompton Europe B.V-Uniroyal Chemical.

in this particular strain (normally responding to frameshift mutations) upon exposure to a compound such as UDMH (see below, where DNA methylation was reported *in vivo*).

- ***DNA FRAGMENTATION IN VITRO***

DNA strand breakage *in vitro* was tested by Sina *et al.* (1983) in rat hepatocytes. The purity of the test material was not stated. There was an inverse dose-response: ++ at 0.03 mM, + at 0.3 mM, and - at 3mM.

- ***MUTATIONS AT THE GENE LEVEL IN MAMMALIAN CELLS***

In the studies on gene mutation in mouse lymphoma L5178Y cells at the thymidine kinase locus (TK +/-), positive results in the absence and the presence of metabolic activation systems (S9 mix) were described by Brusick and Matheson (1976). In the study by Rogers and Back (1981) mutation was observed at the TK locus, but not at the ouabain resistance or cytosine arabinoside resistance loci.

One study mentioned a positive result at the HPRT locus of Chinese Hamster V79 lung cells (Beije *et al.* ,1984) and equivocal results were found by Stankowski and Tunman (1987) and inconclusive results by Stankowski (1988) on Chinese hamster ovary cells.

Apart from the studies of Stankowski and Tunman (1987) and Stankowski (1988), the adoption of protection against UDMH oxidation is unknown in all the other mentioned studies.

- ***MUTATIONS AT THE CHROMOSOMAL LEVEL***

In the only published *in vitro* chromosomal aberration test performed, in Chinese Hamster Ovary (CHO) cells, there were positive results in both the absence and the presence of metabolic activation systems (S9 mix) (JETOC, 1997). Purity and protection of the tested material are unknown.

Another *in vitro* chromosomal aberration test in Chinese Hamster Ovary (CHO) cells with UDMH pure and protected against oxidation, gave negative results (SanSebastian, 1986).

- ***UNSCHEDULED DNA SYNTHESIS***

Two studies, one in male F344 rat primary hepatocytes and the other in ACI/N rat primary hepatocytes, in the absence of exogenous metabolic activation, were negative (Barfknecht, 1986; Mori *et al.*, 1988). A study on C3HeN mouse primary hepatocytes, in the absence of

exogenous metabolic activation, was positive at the highest tested concentration (1 mM), but purity of the test material and protection against oxidation were not stated (Mori *et al.*, 1988).

1.3.2 *In vivo* assays

Available studies include: micronucleus studies on mouse bone marrow cells, hepatocytes, splenocytes and spermatids; a mouse DNA fragmentation test; an unscheduled DNA synthesis test in rat kidney cells; and dominant lethal tests.

In general the PPR Panel noted that there is poor consistency of results of the *in vivo* assays. The weighted evaluation of the *in vivo* assays is difficult because of differences in endpoints and tissues selected and because of technical limitations of some studies.

It was noted that there were consistently negative results from micronucleus tests performed on mouse bone marrow cells in three independent laboratories (Bruce and Heddle, 1979; Cliet *et al.*, 1993; Suzuki *et al.*, 1994). However, one laboratory obtained positive micronucleus test results in spermatids and hepatocytes of similarly treated mice (Cliet *et al.*, 1989 and 1993; protection of UDMH not stated). A sperm abnormality test was negative in male mice tested with 500 mg/kg bw (Bruce and Heddle, 1979), even though the authors used a test material of doubtful purity as it provided an unusually high LD50. Another laboratory obtained a positive micronucleus test result using mouse splenocytes, but without dose-response (Benning *et al.*, 1994).

Thus, all of the micronucleus studies indicate that bone marrow is not a target. Other tissues (splenocytes, hepatocytes, spermatids) seem to be possible targets, but uncertainty about purity and protection against oxidation of the test material and absence of result confirmation by independent laboratories make the evaluation uncertain.

A published report on DNA fragmentation (Parodi *et al.*, 1981) considered the results found in mouse liver after single and repeated dosing as negative, while the results observed in mouse lung after repeated dosing only were considered to be positive. However, the tested doses were higher than (the single dose) or not far from (the repeated dose) the LD50, thus questioning the validity of the results.

All of the aforementioned studies used mice dosed by the i.p. route.

The COMET assay was used by Sasaki *et al.,* (1998) in male mice dosed by intraperitoneal (i.p.) injection at 50 mg/kg bw or with oral doses of 50 or 100 mg/kg bw. Positive results were obtained after 3 hr in liver and lung and at 24 hr in lung with the i.p. administration and in liver, lung, stomach and colon with the oral administration at 3 hr. The kidney, bladder, bone marrow, and brain were always negative. Protection of the test material against oxidation was not stated.

Two dominant lethal tests, one in ICR/Ha Swiss mice *in vivo* and another in mice *in vivo* were both negative (Epstein *et al.*, 1972; Brusick and Matheson, 1976).
An unscheduled DNA synthesis test in Fischer 344 rat kidney cells *in vivo* was also negative (Tyson and Mirsalis, 1985).

It was also noted that in the only published study of its type, N7-methylation of guanine was found following oral administration of UDMH to rats (Sagelsdorff *et al.*, 1988). This indicates that UDMH can react covalently with DNA *in* vivo and therefore could have the potential to be genotoxic. It was found that following daminozide, the extent of N7-alkylation was approx. 50-fold less. It should be noted, however, that with respect to mutagenicity, methylation of the O6 of guanine is the key event, it having been shown that such adducts are the most important in terms of premutagenic lesions. Moreover, the ratio of methylation between N7 and O6 may differ not only between compounds but also between target tissues. Therefore the interpretation of the significance of these data is uncertain.

1.3.3 Evaluation of UDMH genotoxicity by PPR Panel

Based on the results available concerning *in vitro* studies carried out with UDMH protected against oxidation (no *in vivo* studies are available), the PPR Panel concluded that protected UDMH did not show any evidence of genotoxic action.

Looking at the results of *in vitro* and *in vivo* studies concerning unprotected UDMH or UDMH of unspecified protection, the PPR Panel concluded that they provide patchy, limited evidence of a possible genotoxic potential of UDMH both in prokaryotes and eukaryotes. The evidence resides in a weak mutagenic activity evidenced in 2 studies on *Salmonella typhimurium* TA98; two positive studies in mouse lymphoma L5178Y cells; one study showing chromosomal aberrations in Chinese Hamster Ovary (CHO) cells; an *in vivo* study showing micronucleus induction in mouse hepatocytes and spermatids; and an *in vivo* study showing DNA fragmentation (COMET assay) in the liver and lung of male mice. These positive studies, however, were not duplicated by independent laboratories and, as discussed above in detail for each study, some of them suffered some technical limitations.
The PPR Panel noted that when UDMH in solution was left exposed to open air for 19 hr, it converted from negative to positive on S. typhimurium TA1530 and TA1535 with and without metabolic activation, whereas it remained negative on TA100 (Lunn *et al.*, 1991). In the same experiment NDMA was positive only on TA100 and only with metabolic activation. These results, while emphasising the importance of possible formation of active mutagenic

derivatives on open air oxidation of UDMH, seem also to indicate that NDMA may not be the only active derivative formed.

1.4 Carcinogenicity studies of daminozide

Several carcinogenicity studies on daminozide are available in rats and mice.

Two long-term studies in rats (NCI, 1978) and mice (NCI, 1978; Toth *et al.*, 1977) on daminozide were carried out in the seventies.

In the study reported by Toth in 1977, the maximum tolerated dose (MTD) was clearly exceeded at 20,000 ppm of daminozide based on the presence of significant toxicity and mortality. The control animals in the Toth study were reported to be from an earlier study and the purity of the test material was not given.

The NCI rat (Fischer 344) and mouse (B6C3F1) bioassays reported no increase in tumors in male rats or female mice. There was a slight increase in uterine tumors in rats that was not statistically significant, and the increase in liver tumors in male mice was not considered significant because of the high spontaneous rate and variability of this tumor type.

In the more recent studies conducted according to Good Laboratory Practice (GLP) standards (Johnson, 1988), where the purity of the test material (daminozide) was known, no statistically significant increase in tumour incidences was reported. These two studies used daminozide technical material of known purity, containing typical amounts of the impurity UDMH (~30 ppm). No significant oncogenic effects were found in either Fischer 344 rats or CD-1 mice fed up to 10,000 ppm (corresponding respectively to about 500 and 1,500 mg daminozide/kg bw per day).

The PPR Panel concluded that these studies do not provide any evidence that daminozide induces carcinogenic effects in rats and mice.

As discussed above, a significant proportion of the administered daminozide dose in the male rat is expected to have been metabolically converted into UDMH: conservatively, metabolically formed UDMH can be estimated to represent at high doses at least 10% of the administered dose. Thus these studies indirectly suggest that metabolically-formed UDMH was ineffective in causing carcinogenic effects in male rats up to an approximate dose of 50 mg/kg bw per day. If a similar metabolic pattern of conversion of daminozide into UDMH is also valid for female rats and mice, these carcinogenicity studies on daminozide would indicate that metabolically formed UDMH is ineffective in causing carcinogenic effects in female rats up to about 50 mg/kg bw per day and in mice up to about 150 mg/kg bw per day. Such indications are in contradiction with the results of the carcinogenicity studies of UDMH in treated female rats starting from a dose of 4.5 mg/kg bw per day and in mice starting from a dose of 2.7 mg/kg bw per day (see below).

Several different untested hypotheses could explain this discrepancy, including a markedly different metabolic pattern between male and female rats and between male rats and mice, or a different toxicological behaviour between metabolically-formed and orally-administered UDMH. This toxicological uncertainty confounds the interpretation of the carcinogenic potential of UDMH.

1.5 Carcinogenicity studies on UDMH

Three carcinogenicity studies on UDMH are available, one in rats and two in mice.

1.5.1 Rat Study

Seventy male and 70 female Charles River Fischer 344 were given UDMH at concentrations of 0, 1, 50, 100 ppm in deionised tap water corresponding to 0, 0.07, 3.2, 6.2 mg/kg bw/day for males and 0, 0.1, 4.5, 7.9 mg/kg bw/day for females (calculated as average daily intake throughout the duration of the study) for 24 months. Test article (100 mg/ml in 1 N HCl) was stored at room temperature under nitrogen headspace. Food and water were available *ad libitum* (Goldenthal, 1989a).

Statistically significant but unimportant reductions in body weight (range 2-5%) were detected at 100 ppm in males and both at 50 and 100 ppm in females. No differences in food intake were detected. Scattered reductions in water intake were noted in UDMH treated rats at all concentrations; reduced water intake was more consistent at 50 and 100 ppm, in males during the last 20 weeks of the study and in females throughout the entire study.

No haematological effects were detected at any time. No signs of UDMH related toxicity were noted in any treated group during the study. At the end of the treatment, the mortality rate was 36%, 36%, 28%, 18% (males) and 32%, 24%, 28%, 10% (females) at 0, 1, 50, 100 ppm UDMH, respectively.

At the 12-month sacrifice, there were no macroscopic or microscopic treatment-related lesions. At the 24-month sacrifice, gross pathological effects were comparable between groups except for the incidence of cloudy corneas (slightly increased in females at 50 and 100 ppm with respect to controls - 37% and 41% instead of 27%). This macroscopic alteration corresponded histologically to a higher incidence of corneal mineralisation. There was no morphological evidence of treatment-related hepatotoxicity.

The incidence of pituitary adenoma was increased in females at 100 ppm (56%) in comparison to that in controls (32%). The incidence of total hepatocellular neoplasms was increased in female rats at 50 ppm (10%) and 100 ppm (10%) with respect to controls (0%) but not in male rats. In female rats the incidence was 0%, 2%, 4% and 2% for hepatocellular adenoma and 0%,

0%, 6% and 8% for hepatocellular carcinomas at 0, 1, 50 and 100 ppm UDMH, respectively. The historical incidence of hepatocellular neoplasms in female Fischer rats at IRDC in 2-year chronic studies is 0.5% (2 adenomas/370 rats) which agrees with the low incidence observed in the present study.

The PPR Panel concludes that the increase in hepatocellular carcinomas, a rare neoplasm in female F344 rats at doses almost devoid of other toxic effects, suggests an oncogenic effect of UDMH in female rats. The incidence of pituitary adenomas was also increased in females at 100 ppm, but the toxicological relevance of this finding is uncertain. The highest dose without detectable effects in this study is 1 ppm UDMH in drinking water corresponding to 0.09 mg/kg bw/day.

1.5.2 Other studies related to carcinogenicity in rats

The PPR Panel noted the negative results of a study on carcinogenic potential of daminozide and UDMH in a shorter term *in vivo* bioassay system in rats (Cabral *et al.*, 1995). This study examined the carcinogenic potential of daminozide alone and in combination with UDMH in a medium-term bioassay in Fischer 344 rats in the DEN (N-nitrosodiethylamine)-PH (partial hepatectomy) model.

Neither daminozide alone (20,000 ppm in diet) nor daminozide (20,000 ppm) in combination with UDMH (75, 150, 300 ppm) induced an increase in the number and/or size of GST-P positive foci in the liver. Also UDMH alone, tested at levels of 75, 150 or 300 ppm in the diet, was negative in this experiment. According to some interpretations, these results would imply that neither daminozide, UDMH nor the combination has carcinogenic potential.

The PPR Panel agrees that positive results in the DEN-PH model have a high positive predictive value for liver carcinogenicity in rats and mice, but is of the opinion that the negative predictivity of this model is uncertain and requires further investigation. Moreover the observation of negative results in male rats is consistent with the negative results observed in the long term carcinogenicity testing in this species and gender, but does not add relevant information on the possible effects in female rats or in mice.

Therefore it is the opinion of the PPR Panel that the study of Cabral *et al.* (1995) cannot be used to override the carcinogenic effects on liver observed in the chronic studies with UDMH in female rats.

1.5.3 Mouse Studies

- *LOW DOSE STUDY*

Ninety male and 90 female Charles River CD-1 mice (Charles River Breeding Laboratories, approximately 6 weeks old at the beginning of the study) were given UDMH at concentrations of 0, 1 or 5 ppm in deionised tap water (with 25% citrate buffer to neutralize acidic pH) equal to 0, 0.19, 0.97 mg/kg bw/day for males and 0, 0.27, 1.4 mg/kg bw/day for females (calculated as average daily intake throughout the duration of the study) for 24 months. Another 90 mice per group received UDMH at concentrations of 10 ppm (males) and 20 ppm (females), equal to 1.9 and 2.7 mg/kg bw/day, respectively. Test article (100 mg/ml in 1 N HCl) was supplied by the producer in 26 different specimens containing 93 to 115% of the claimed concentration (range of concentrations after being analyzed in the same laboratory). Test article was stored at room temperature under nitrogen headspace. Drinking water containing the test article was prepared and changed three times a week (stable up to three days at all concentrations). The actual content of UDMH in the solutions was checked twice monthly and showed 102%, 100%, 100%, 101% of nominal concentrations (mean of 51 determinations) for 1, 5, 10, 20 ppm, respectively. Food and water were available *ad libitum* (Goldenthal, 1989b).

No consistent dose-related differences in body weight were detected throughout the study in any group. All groups showed similar daily food and water intakes.

At 6, 12, 18, 24 months from the beginning of the study, the following haematological tests were performed in 10 animals/sex group; leukocyte count, erythrocyte count, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets, differential leukocyte count. At 24 months from the beginning of the study the following biochemical tests were performed in 10 animals/sex/group: total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase.

Statistically but not biologically significant differences of some haematological parameters were detected at the 18 month interval in female mice at 5 and 20 ppm. At 24 months these differences were no longer evident.

There were no toxic effects detectable on biochemical tests at the 24 month time point. At the end of treatment the mortality rate was 48%, 54%, 52% and 68% in males in the 0, 1, 5, 10 ppm dose groups and 60%, 50%, 64% and 76% in females in the 0, 1, 5, 20 ppm dose groups. The mortality rate was significantly increased only in males at 10 ppm.

Pathology was performed at spontaneous death and at 8 and 12 months (20 mice/sex/group) and at 24 months (remaining animals) from the beginning of the study. Gross pathology showed an increased incidence of macroscopic masses/nodules in the lung for the 12 to 24

month period in males at 5 ppm (47%) but not at 10 ppm (26%) in comparison to controls (21%) and in females at 20 ppm (43%) in comparison to controls (12%).

Histopathological examination showed a variety of non-neoplastic and neoplastic lesions in both sexes across dose levels.

A dose-related increase of brown pigment in the liver was noted in both sexes. Although special stains were not performed to determine the specific type of pigment, it appeared to be of the ceroid or lipofuscin pigment type, sometimes haemosiderin or bile pigment. Brown pigment was also present in some male mice at the highest dose at the 8 and 12 month sacrifice but not in other groups.

The incidence of haemangiosarcomas in females was 3/49, 2/48, 1/48 and 5/49 in the 0, 1, 5, 20 ppm dose groups.

The incidence of pulmonary neoplasms in females at the 20 ppm concentration was increased. Alveolar/bronchiolar adenomas were found in 20/49 dosed mice versus 5/49 in controls and alveolar/bronchiolar carcinomas were 7/49 versus 1/49. These differences were statistically significant and a positive statistical trend for pulmonary neoplasms was found.

The incidence of these neoplasms in female controls was in the range of historical controls at the testing laboratory (for adenomas 6/69 and for carcinomas 2/69).

The incidence of pulmonary neoplasms was not increased in male mice up to 10 ppm UDMH. The statistically significant increase of mortality rate in males at 10 ppm may be incidental because other clinical or morphological signs of toxicity were lacking in these animals. The mortality rate is not different from that calculated in controls in a subsequent study performed in the same strain of animals by the testing laboratory.

The PPR Panel concludes that this study provides evidence of an increased incidence of lung tumours in females at 20 ppm UDMH, corresponding to 2.7 mg/kg bw/day. The highest dose without detectable effects in the present study is 5 ppm corresponding to 1.41 mg/kg bw/day.

- ***HIGH DOSE STUDY***

Ninety male and 90 female Charles River CD-1 mice (Charles River Breeding Laboratories), approximately 6 weeks old at the beginning of the study were given UDMH at concentrations of 0, 40 and 80 ppm in deionised tap water (with 25% citrate buffer to neutralise acidic pH) equal to 0, 7.34, 13.0 mg/kg bw/day for males and 0, 11.6, 21.8 mg/kg bw/day for females (calculated as average daily intake throughout the duration of the study) for 24 months. Test article was the same as supplied for the previously cited study and was stored in the same

conditions. The actual content of UDMH in the solutions was checked twice monthly and resulted in 103% and 102% of nominal concentrations (mean of 54 determinations) for 20 and 40 ppm, respectively. Food and water were available *ad libitum* (Goldenthal, 1990).

The small number of surviving animals in the highest dose groups and the great variability in the data between weeks hampered evaluation of mean body weight, water and food consumption. No consistent dose-related differences in body weight were detected throughout the duration of the study at 40 ppm for either sex nor at 80 ppm in males. Body weight was reduced approximately 10% in females at 80 ppm during the last 6 months of UDMH administration. Scattered significant reductions in daily food intake suggest reduced average food consumption for both sexes at the highest dose during the last months of the study. Water consumption was reduced at both UDMH concentrations for the duration of the study in males and only during the first 13 weeks in females.

A dose-related effect on some haematological parameters was detected in males, but not in females (except at 24 months when the small number of survivors hampered statistical evaluation), starting 6 months (80 ppm) and 12 months (40 ppm) from the beginning of the study.

A significant but not dose-related increase of alanine aminotransferase and sorbitol dehydrogenase was measured at 12 months in both sexes at both concentrations.

At the end of the treatment period, the mortality rate was 70%, 76%, 98% in males and 58%, 92%, 92% in females in the 0, 40, 80 ppm dose groups, respectively. The mortality rate was significantly increased in both sexes at the highest dose.

Gross pathology showed accentuated liver lobulation in male mice but not in females at both dose levels. Macroscopic nodules in the lungs and nodules/masses in the liver were increased in males from 8 months onwards and in females from 12 months onwards at both dose levels. Histopathological examinations showed several signs of hepatotoxicity, which were: multifocal chronic inflammation (in males at both dose levels prevalent in the 12-24 month section of the study), cell hypertrophy and necrosis (in males at both dose levels detectable in all months of the study), brown pigment (in both sexes detectable from 0-8 month section up to the end of the study at both dose levels). Special stains were performed to identify pigments. Haemosiderin, bile pigments, ceroid and lipofuscin, the amount of collagen and reticulum were all increased. Increased extramedullary haematopoiesis in the spleen of both species occurred from 12 months onwards.

At the end of the study the incidence of vascular neoplasms in the liver (haemangiomas and haemangiosarcoma) was: 9%, 67% and 81% at 0, 40, 80 ppm in males and 4%, 26% and 82% at 0, 40 and 80 ppm in females (in both sexes the large majority of these tumours – 95% - were hemangiosarcomas). Many of the premature decedents also showed hepatic

haemangiosarcomas. In mice sacrificed/dead during the 8-12 months period of the study, the incidence of alveolar/bronchiolar neoplasms was 18%, 45% and 55% at 0, 40 and 80 ppm in males and 14%, 50% and 48% at 0, 40 and 80 ppm in females. At the end of the study the incidence of these neoplasms was 54%, 73% and 51% at 0, 40 and 80 ppm in males and 31%, 53% and 56% at 0, 40 and 80 ppm in females.

The PPR Panel noted that high doses of UDMH induced increased incidences of neoplasms of the lung and of the vasculature of the liver in mice. The PPR Panel also noted that the carcinogenic effects occurred at doses possibly exceeding the maximum tolerated dose, which caused overt toxicity in exposed mice. A dose without effect cannot be identified from this study.

1.6 Conclusions of the PPR Panel on UDMH carcinogenicity

Based on the toxicological studies in rats, the PPR Panel concluded that while there is no evidence of carcinogenicity in male rats at least up to 6.2 mg/kg bw/day, there is an indication of carcinogenicity in female rats starting at a dose of 4.5 mg/kg bw per day. The highest dose without detectable effects in rats is 1 ppm UDMH in drinking water corresponding to 0.09 mg/kg bw/day.

In mice, administration of UDMH in drinking water was associated with an increased incidence of pulmonary neoplasms in females at 20 ppm (corresponding to 2.7 mg/kg bw per day) but not at 5 ppm (equal to 1.4 mg/kg bw/day) or in male mice up to 10 ppm (equal to 1.9 mg/kg bw per day). At higher doses of 40 and 80 ppm, neoplasms of the lung and the vasculature of the liver occurred in both sexes, but the PPR Panel noted that hepatotoxicity occurring at these high doses complicated the interpretation of these findings.

On the whole, the available studies indicate a carcinogenic potential of UDMH both in female rats and in mice in a range of doses of a few mg/kg bw per day. In both cases doses without detectable effects (1.4-0.09 mg/kg bw per day) were determined.

The PPR Panel noted that the results provided by direct long term testing of UDMH in rodents are in apparent discrepancy with those obtained by testing daminozide. In fact, the daminozide long term studies were negative in rats and mice at doses that should have produced internal doses of metabolically-formed UDMH at least one order of magnitude higher than those proven to be carcinogenic on direct testing. While, as noted before, a possible explanation for such a discrepancy could reside either in a different intrinsic carcinogenic potential between the metabolically-formed UDMH and the UDMH used for the carcinogenicity testing or in a different metabolic behaviour of UDMH in female rats and mice, the PPR Panel is not in a position to judge the plausibility of such explanations, due to the limited knowledge available on UDMH

bioavailability and metabolism. The PPR Panel did note that oral UDMH was almost 50-times more effective than daminozide at the N7-methylation of guanine residues in DNA in the male rat, although the significance of this is unclear. These toxicological inconsistencies add uncertainty to the interpretation of the carcinogenic potential of UDMH.

On balance, the PPR Panel believes that the weight of evidence is against a genotoxic mechanism for the carcinogenic effects of UDMH because there is only patchy, limited evidence of genotoxic potential of UDMH (see section 1.3.3).
Since specific investigations on non-genotoxic mechanisms, for example on cell proliferation and hormonal imbalance, are not available for UDMH, a more definitive conclusion on the mechanism involved is not possible at the moment.

CONCLUSIONS AND RECOMMENDATIONS

The Scientific Panel on Plant health, Plant protection products and their Residues, having regard to the submitted question, concludes that, on the basis of the available data, it is not possible to identify the mechanism responsible for the carcinogenic action of UDMH in rodents. There is no *in vitro* evidence of genotoxicity of pure and oxidation-protected UDMH and *in vivo* studies on this are not available. The PPR Panel noted an apparent discrepancy in that daminozide long-term studies did not give rise to carcinogenicity in rats and mice at doses that should have produced internal doses of metabolically-formed UDMH at least one order of magnitude higher than those proven to be carcinogenic on direct testing. In addition, N7-methylation of guanine was found to be 50-fold higher in a study following oral administration of UDMH to rats, when compared to the corresponding data for daminozide. Thus the PPR Panel considered that any conclusion on the mechanism of carcinogenicity of orally administered UDMH should be regarded to include a margin of uncertainty. The PPR Panel concluded that the weight of evidence is against a genotoxic mechanism.
Among possible non-genotoxic mechanisms, altered regulation of cell proliferation or hormonal imbalance are plausible alternatives to genotoxicity, but these mechanisms have not been specifically investigated and thus a more definitive conclusion on the mechanism involved is not possible at the moment.
In experimental testing of UDMH for carcinogenicity in rats and mice, no effects were observed at 0.09 mg/kg bw/day and 1.41 mg/kg bw/day, respectively.
If the observed UDMH carcinogenicity is due to a non-genotoxic mechanism, the above indicated doses should be considered to be toxicological thresholds. However, taking together the uncertainties associated with the mechanism and the possibility that UDMH in greenhouse conditions may form oxidised derivatives (as mentioned in the background of this opinion) that

might be genotoxic, the PPR Panel is of the opinion that any use of these doses as thresholds should be undertaken only with due caution.

DOCUMENTATION PROVIDED TO EFSA

(*see also additional references in list of references*)

1. Letter from Mr A. Checchi-Lang from the Health & Consumer Protection Directorate-General requesting a consultation EFSA on daminozide, with ref. OLD\daminozide (330)\consut-Scientific Panel (rev 1)–D/511793, 22 December 2003.
2. Letters Fine Agrochemicals Ltd. 19 September 2003, pp 1-3 and 18 December 2003, pp 1-2.
3. Letter from the Danish EPA : comments on ECCO 134, 05 January 2004, p 1.
4. Letter Crompton/Uniroyal Chemical to EFSA, 19 December, 2003, p1.
5. Monograph[8] on daminozide–Volume 3-Annex B–B6-Toxicology and metabolism–June 1999, pp 46-171.
6. Monograph on daminozide –Addendum - Volume 3-Annex B – June 2002, pp 1-77.
7. Monograph on daminozide –Addendum - Volume 3-Annex B – B6-Toxicology and metabolism – June 2003, pp 1-11.
8. Monograph on daminozide –Addendum III - Volume 3-Annex B – B6-Toxicology and metabolism – ECCO 131- December 2002, pp 1-4.
9. Report from ECCO 131- 12 February 2003 + evaluation table Doc. SANCO/3042/99 rev.4-1, 12 February 2003, pp 1-34.
10. Evaluation table Doc. SANCO/3042/99 rev.7, 07 November 2003, pp 1-64.
11. Final report on *in vivo* micronucleus and chromosome aberration assay in mouse bone marrow cells. pp 1-51.
12. Monograph on daminozide–Volume 2-Annex A–June 1999, pp 1-32.
13. Monograph on daminozide–Volume 1-Report and proposed decision–June 1999, pp 1-87.
14. Monograph on daminozide–Volume 1-Addendum-Report and proposed decision–June 2002, pp 1-90.
15. ACGIH-American Conference of Governmental Industrial Hygienists, 1991. Documentation of the threshold Limit Values and Biological Exposure Indices, 6th Ed Vol. Cincinnati, Ohio, pp 491-494.
16. Archer M.C. and Eng V.W.S., 1981, Quantitative determination of carcinogens and mutagens as alkylating agents following chemical activation. Chem.-Biol Interactions, 33, 207-214.
17. Barfknecht T.R., 5 December, 1986, Rat Hepatocyte Primary Culture/DNA Repair Test. Pharmakon Research International, Inc. Pennsylvania USA, pp 1-61.
18. Bartsch H., Malaveille C., Camus A.-M., Martel-Planche G., Brun G., Hautefeuille A., Sabadie N. and Barbin A., 1980, Validation and Comparative Studies on 180 chemicals with S. typhimurium Strains and V79 Chinese Hamster Cells in the Presence of Various Metabolizing Systems. Mutation Research 76, 1-50.
19. Beije B., Onfelt A. and Olsson U., 1984, Influence of Dietary Selenium on the Mutagenic Activity of Perfusate and Bile from Rat Liver, Perfused with 1,1–dimethylhydrazine. Mutation Research, 130, 121-126.

[8] Monograph = draft assessment report

20. Benning V., Brault D., Duvinage C., Thybaud V. and Melcion C., 1994, Validation of the *in vivo* CD1 mouse splenocyte micronucleus test. Mutagenesis 9, (3), 99-204.
21. Bignami M., Conti G., Crebell R. and Carere A., 1981, Growth-mediated metabolic activation of promutagens in Aspergillus nidulans. Mutation Research 80, 265-272.
22. Bootman J. and Lodge D.C., 21 January, 1983, Amended final report, P 7642 Assessment of its ability to induce genetic damage in saccharomyces cerevisiae, Uniroyal Chemical UK, pp 1-19.
23. Bootman J., Rees R. and Anderson C, 13 September, 1982, Final report, P 7642 Investigation of mutagenic activity in the TK+/- Mouse Lymphoma cell mutation assay. Uniroyal Chemical UK, pp 1-26.
24. Bruce R.W. and Heddle J.A., 1979, The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. Can. J. Genet. Cytol., 21, 319-334.
25. Brusick D. and Matheson D., 1976, Mutagenic evaluation of 1,1-dimethylhydrazine, methylhydrazine and N-Phenyl-α-Naphthylamine. Proceeding of the 7th annual conference of environmental toxicology 13,14 & 15 October 1976. Erospace Medical Research Laboratory Ohio, USA, pp 108-128.
26. Cabral R., Hakoi K., Hoshiya T., Hasegawa R. and Ito N., 1995, Lack of carcinogenicity of Daminozide, alone or in combination with its contaminant 1,1-dimethylhydrazine, in a medium-term bioassay. Teratogenesis, Carcinogenesis, and Mutagenesis, 15, 307-312.
27. Cardona R.A., 4 December, 2001, Discussion of the genotoxicity / carcinogenicity of UDMH. Uniroyal Chemical, The Netherlands, pp 1-46.
28. Cliet I., C. Melcion and Cordier A. 1993, Lack of predictivity of bone marrow micronucleus test versus testis micronucleus test : comparison with four carcinogens. Mutation Research 292, 105-111.
29. Cliet I., Fournier E., Melcion C. and Cordier A., 1989, *In vivo* micronucleus test using mouse hepatocytes. ® Elsevier Science Publishers B.V. pp 321-326.
30. Clive D., McCuen R., Spector J.F.S., Piper C. and Mavournin K.H., 1983, Specific gene mutation in L5178Y cells in cluture. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Research, 115, 225-251.
31. de Flora S. and Mugnoli A., 1981, Relationships between mutagenic potency, reversion mechanism and metabolic behavior within a class of chemicals (hydrazine derivatives). Cancer Letters, 12, 279-285.
32. de Flora S., 1981, A spiral test applied to bacterial mutagenesis assays. Mutation Research 82, 213-227.
33. de Flora S., 1981, Study of 106 organic and inorganic compounds in the Salmonella/microsome test. Carcinogenesis, 2, (4), 283- 298.
34. de Flora S., Zanacchi P., Camoirano A., Bennicelli C. and Badolati G.S., 1984, Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Research, 133, 161-198.
35. Dynamac Corporation Study, Experimental Pathology Laboratories Inc., (MD, USA) Amended Pathology report 21 November, 1988. Two Year Oncogenicity Study in Mice with UDMH. pp 1-149.
36. Dynamac Corporation study, Experimental Pathology Laboratories Inc., (MD, USA) Pathology report 12 December 1988. Two year dietary oncogenicity study in mice with Alar, pp 1-151.
37. Epstein S.S., Arnold E., Andrea J., Bass W. and Bishop Y., 1972, Detection of chemical mutagens by the dominant lethal assay in the mouse. Tox. and applied pharmacology, 23, 288-325.
38. Fathulla R.N.,22 December 1999, Final Report. Study on pharmacokinetics, biliary excretion, mass balance and tissue distribution of [^{14}C-methyl]-daminozide following a single oral dose in male rats. Covance Laboratory, Inc. Wisconsin USA, pp 1-187.
39. Fine proprietal study 18 July 1990 –Evaluation of the ability of Dazide to induce chromosome aberrations in cultured peripheral Human Lymphocytes. pp 1-27.

40. Fine proprietal study 19 Mars 1984– Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of daminozide. pp 1-11.
41. Fine proprietal study 23 March 1989 – The Effects of Dazide on Mutation at the HGPRT Locus of cultured Chinese Hamsters Ovary. pp 1-33.
42. Furihata C. and Matsushima T., 1987, Use of *in vivo/in vitro* unscheduled DNA synthesis for identification of organ-specific carcinogens. CRC Critical Reviews in Toxicology, 17, (3), 245-277.
43. Goldenthal E. I., 28 September, 1989 b, Two year oncogenicity study in mice. International Research and Development Corporation Michigan USA, pp 1-2076.
44. Goldenthal E.I., 31 January 1990, Two year oncogenicity study in mice, International Research and Development Corporation Michigan USA, pp 1-1738.
45. Goldenthal E.I, 23 January, 1992, Historical control data from 2 year rat studies with Fischer rats, bile duct hyperplasia. International Research and Development Corporation Michigan USA, pp 1-1871.
46. Goldenthal E.I., 7 May 2001, Historical control data relevant to the evaluation of tumor incidence in the mouse study. Uniroyal Chemical Europe B.V. (The Netherlands), pp 1-10.
47. Goldenthal Edwin I., 28 September, 1989a, UDMH Rat Chronic 399-062, Two Year Oncogenicity Study in Rats. International Research and Development Corporation Michigan USA, pp 1-1762.
48. Gudi R. and Krsmanovic L., 22 May, 2003, *In vivo* micronucleus and chromosome aberration assay in mouse bone marrow cells. BioReliance MD USA, pp 1-51.
49. Hasegawa R. and Ito N., 1992, Liver medium-term bioassay in rats for screening of carcinogens and modifying factors in hepatocarcinogenesis . Fd. Chem. Toxic. 30, (11), 979-992.
50. Hayes A.W. 2003. An assessment of daminozide and UDMH, A.W., 17 December 2003, pp 1-50.
51. Hemminki K., Falck K. and Vainio H., 1980, Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals epoxides, glycidyl ethers, methylating and ethylating agents, halogenated hydrocarbons, hydrazine derivatives, aldehydes, thiuram and dithiocarbamate derivates. Arch Toxicol., 46, 277-285
52. Ho Y.L. and Ho S.K., 1981, Screening of carcinogens with the prophage λcl ts857 induction test. Cancer Research, 41, 532-536.
53. Jenssen D. and Ramel C., 1980, The micronucleus test as part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents tested. Mutation Research, 75, 191-202.
54. JETOC 1997. Compiled under the supervision of Chemical Substance Investigation Division. Mutagenicity test data of existing chemical substances based on the toxicity investigation system of the industrial safety and health low supplement. Industrial Safety and Health Department, Labour Standards Bureau, Ministry of Labour, Japan, Ed. Japan Chemical Industry Ecology-Toxicology & Information Center JETOC, pp 1-4.
55. Johnson D.E., 29 August, 1988, Two year dietary Oncogenicity Study in Mice. International Research and Development Corporation Michigan USA, pp 1-1563.
56. Klopman G., Frierson M.R. and Rosenkranz H.S., 1990, The structural basis of the mutagenicity of chemicals in Salmonella typhimurium : The gene-tox data base. Mutation Research, 228, 1-50.
57. Krüger F.W., Wiessler M. and Rücker U., 1969, Short Communications Investigation of the alkylating action of 1,1-dimethylhydrazine. Biochemical Pharmacology, 19, 1825.
58. Lamb J. C. and Hauswirth J., 1994–Risk Assessment of daminozide and UDMH to Support Registration.pp 1-15.

59. Lunn G. and Sansone E. B., 1994. Oxidation of 1,1–dimethylhdrazine (UDMH) in Aqueous Solution with Air and Hydrogen Peroxide. Chemosphere, 29 (7), pp 1577-1590.
60. Lunn G., Sansone E. B. and Andrews A.W., 1991. Aerial Oxidation of Hydrazines to Nitrosamines, Environment Molecular Mutagenesis, 17, pp 59-62.
61. Matsushita H. Jr., Endo O., Matsushita H., Yamamoto M. and Mochizuki M., 1993, Mutagenicity of alkylhydrazine oxalates in Salmonella typhimurium TA100 and TA102 demonstrated by modifiying the growth conditions of the bacteria. Mutation Research, 301, 213-222.
62. Medical Health Surveillance on daminozide Manufacturing plant Personnel. 22 Septembre 1999. Report SNPE Chimie, Sorgues, France. pp 1-4.
63. Mori H., Sugie S., Yoshimi N., Iwata H., Nishikawa A., Matsukuho K., Shimizu H. and Hirono I., 1988, Genotoxicity of a variety of hydrazine derivatives in the hepatocyte primary culture/DNA repair test using rat and mouse hepatocytes. Jpn J. Cancer Res. (Gann), 79, 204-211
64. NatajaranA.T. and Obe G., 1986, How do *in vivo* mammalian assays compare to *in vitro* assays in their ability to detect mutagens ? Mutation Research, 167, 189-201.
65. Nielsen P.A., Lagersted A., Danielsen S., Jensen A.A., Hart J. and Larsen J.C., 1992, Mutagenic activity of nine N, N-disustituted hydrazines in the Salmonella / mammalian microsome assay. Mutation Research, 278, 215-226.
66. Parodi S., de Flora S., Cavanna M., Pino A., Robbiano L., Bennicelli C. and Brambilla G., 1981, DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. Cancer Research, 41, 1469-1482.
67. Poso A., von Wright A. and Gynker J., 1995, An empirical and theoretical study on mechanisms of mutagenic activity of hydrazine compounds. Mutation Research vol. 332 © Elsevier Science B.V., pp 63-71
68. Putman D.L. and Morris M.J., 20 February, 1991, Final Report. Study on Chromosome aberrations in Chinese Hamsters ovary (CHO) cells. Microbiological Associates, Inc. MD USA, pp 1-37.
69. Rogan E.G., Walker B.A., Gingell R., Nagel D.L. and Toth B., 1982, Microbial mutagenicity of selected hydrazines. Mutation Research, 102, 413-424.
70. Rogers A. M. and Back K.C., 1981, Comparative mutagenicity of hydrazine and 3 methylated derivatives in L5178Y mouse lymphoma cells. Mutation Research, 89, 321-328.
71. Sagelsdorff P., Lutz W.K. and Schlatter C., 1988, DNA methylation in rat liver by daminozide, 1,1-dimethylhydrazine and dimethylnitrosamine. Fundamental and applied toxicology, 11, 723-730.
72. San R. H.C. and Shelton J.B., 21 March, 1991, Amended final report. Study on Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (AMES Test) with a confirmatory Assay. Microbiological Associates, Inc. MD USA, pp 1-70.
73. San R.H.C., Shelton J.B.and Putman D.L., 7 May, 2001, Mutagenicity studies with daminozide : levels of impurities UDMH and NDMR. Uniroyal Chemical Europe B.V. The Netherlands, pp 1-11.
74. Sans, R.H.C., Shelton J.B. and Putman D.L., May 7, 2001, Mutagenicity studies with daminozide : levels of impurities UDMH & NDMA., Uniroyal Chemical Europe B.V., The Netherlands, pp 1-29.
75. SanSebastian J.R., 9 December, 1986, *In vitro* chromosome aberration analysis in Chinese Hamster ovary (CHO) cells, Pharmakon Research International, Inc. Pennsylvania USA, pp 1-33
76. Sasaki Y.F., Saga A., Akasaka M., Ishibashi S., Yoshida K., Su Y.Q., Matsusaka N. and Tsuda S., 1998, Organ-specific genotoxicity of the potent rodent colon carcinogen 1,2-dimethylhydrazine and three hydrazine derivatives : difference between intraperitoneal and oral administration. Mutation Research 415, 1-12.

77. Sedgwick B., 1992, Oxidation of methylhydrazine to mutagenic methylating derivatives and inducers of the adaptive response of Escherichia coli to alkylation damage. Cancer Research, 52, 3693-3697.
78. Sina J.F., Bean C.L., Dysart G.R., Taylor V.I. and Bradley M.O., 1983, Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutation Research, 113, 357-391.
79. Slauter R.W., Coleman D.P. and Jeffcoat A.R. 28 October 1993- Metabolism and Excretion of Daminozide in male rats at a single oral dose level. Fine study. pp 1-104.
80. Stankowski L.F., 9 December, 1986, Ames/Salmonella Plate Incorporation Assay. Pharmakon Research International, Inc. Pennsylvania USA, pp 1-20.
81. Stankowski L.F., 12 September 1988, Unsymmetrical Dimethylhydrazide (UDMH). CHO/HPRT Mammalian Cell Forward Gene Mutation Assay. Pharmakon Research International, Inc. Pennsylvania USA, pp 1-33.
82. Stankowski L.F., 16 January, 1987, CHO/HPRT Mammalian Cell Forward Gene Mutation Assay, UDMH. Pharmakon Research International, Inc. Pennsylvania USA, pp 1-54.
83. Suter W. and Jaeger I., 1982, Comparative evaluation of different pairs of DNA repair-deficient and DNA repair-proficient bacterial tester strains for rapid detection of chemical mutagens and carcinogens. Mutation Research, 97, 1-18.
84. Suzuki Y., Shimizu H., Ishikawa T., Sakaba H., Fukumoto M., Okonogi H. and Kadoruka M., 1994, Effects of prostaglandin E_2 on the micronucleus formation in mouse bone marrow cells by various mutagens. Mutation Research, 311, 287-293.
85. Tosk J., Schmeltz I. and Hoffmann D., 1979, Hydrazines as mutagens in a histidine-requiring auxotroph of Salmonella typhimurium. Mutation Research, 66, 247-252.
86. Travis C.C., Wang L.A. and Waehner M.J., 1991. Quantitative correlation of carcinogenic potency with four different classes of short-term test data. Mutagenesis, 6, (5), 353-360.
87. Tyson C. K. and Mirsalis J.C., 1985, Measurement of unscheduled DNA synthesis in rat kidney cells following *in vivo* treatment with genotoxic agents. Environmental Mutagenesis, 7, 889-899.
88. van der Jagt K.E., Ravensberg J.C., de Wolf J.M. and Links I.H.M., 11 March, 2002, TNO Report on Dislodgeable foliar residue determination of daminozide, UDMH, NDMA and air monitoring of UDMH, NDMA and formaldehyde, following indoor treatment or ornamental plants with ALAR®64 SP. Uniroyal Chemical Europe (The Netherlands), pp 1-48.
89. Vogel E.W. and Nivard M.J.M., 1993, Performance of 181 chemicals in Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis, 8, (1), 57-81.
90. von Wright A. and Tikkanen L., 1980 The comparative mutagenicities of hydrazine and its mono – and di-methyl derivatives in bacterial test systems. Mutation Research, 78, 17-23.
91. Wargovich M.J., Goldberg M.T., Newmark H.L. and Bruce W. R., 1983, Nuclear aberrations as a short-term test for genotoxicity to the colon: evaluation of nineteen agents in mice. JNCI, vol.71, n°1, pp 133-137
92. Watanabe K., Sakamoto K. and Sasaki T., 1996, Comparisons in chemically-induced mutagenicity among four bacterial strains, Salmonella typhimurium TA102 and TA 2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: collaborative study I. Mutation Research, 361, 143-155.
93. Watanabe K., Sasaki T. and Kawakami K., 1998, Comparisons of chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638 and Escherichia coli WP2/ pKM101 and WP2 uvrA/pKM101 : collaborative study III and evaluation of the usefulness of these strains. Mutation Research, 416, 169-181.

94. Waters M.D., Stack H.F., Jackson M.A., Bridges B.A. and Adler I.-D, 1994, The performance of short-term tests in identifying potential germ cell mutagens: a qualitative and quantitative analysis. Mutation Research, 341, 109-131.
95. Weir R.J., 3 February, 1989, daminozide EPA Evaluation record on Alar Chronic toxicity/oncogenicity feeding study in rats, daminozide EPL pathology report on UDMH oncogenicity study in mice, daminozide EPL Amended pathology report on UDMH oncogenicity study in mice, daminozide EPL Pathology report on Alar oncogenicity study in rats, daminozide EPL Pathology report on Alar Oncogenicity study in Mice. Dynamac Corporation MD, USA, pp 1-171.
96. WHO-World Health Organisation, International Agency for Research on Cancer,1999. IARC Monographs in the Evaluation of the Carcinogenic Risks of the Chemicals to Man. Geneva, 71, pp 1425- 1436.
97. Wyrobek A.J. and Bruce W.R., 1975, Chemical induction of sperm abnormalities in mice. Cell Biology Proc. Nat. Acad. Sci. USA, 72, (11), 4425-4429.
98. Zijlstra J.A.and Vogel E.W., 1988, Influence of inhibition of the metabolic activation on the mutagenicity of some nitrosamines, triazenes, hydrazines and seniciphylline in *Drosophila melanogaster*. Mutation Research, 202, 251-267.

REFERENCES

1. NCI-National Cancer Institute, 1978. Bioassay of daminozide for possible carcinogenicity (CAS No. 1596-84-5). National Toxicology Program. Natl. Toxicol. Program Tech. Rep. Ser. 83, pp 1-131.
2. Toth B., 1977. The Large Bowel Carcinogenic Effects of Hydrazines and Related Compounds Occurring in Nature and the Environment. Cancer, 40, 2427-2431.
3. IARC-International Agency Research Cancer, 1999. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide (Part Three). IARC Monographs-1,1-dimethylhydrazine - VOL. 71, pp 1-1425.
4. JMPR-Joint Meeting Pesticide Residues, 1991. Monographs and evaluations 825. Daminozide (Pesticide residues in food: Evaluations (Part II Toxicology).
5. US-EPA 1993 (United States Environmental Protection Agency). Daminozide RED Facts. Office of Prevention, Pesticides and Toxic Substances, EPA-738-F-93-007, September 1993.

SCIENTIFIC PANEL MEMBERS

Jos Boesten, Alan Boobis, Anthony Hardy, Andy Hart, Herbert Koepp, Robert Luttik, Kyriaki Machera, Marco Maroni, Douglas McGregor, Otto Meyer, Angelo Moretto, Euphemia Papadopoulou-Mourkidou, Ernst Petzinger, Kai Savolainen, Andreas Schaeffer, Walter Steurbaut, Despina Tsipi-Stefanitsi, Christiane Vleminckx.

APPENDIX

Table 1. Genetic and related effects of 1,1-dimethylhydrazine (*from International Agency for Research on Cancer (IARC), 1999; re-Evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide (Part Three), Monographs, 71, 1,1-Dimethylhydrazine. pp1425-1436 and documentation provided to EFSA)*

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	NT	–	17000	Ho & Ho (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	250	Bruce & Heddle (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Von Wright & Tikkanen (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	*Stankovski, 1986
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	4800	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	De Flora *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	(+)	NT	5000	Tosk *et al.* (1979)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	15000	Bartsch *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bruce & Heddle (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	4800	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Rogan *et al.* (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	De Flora *et al.* (1984)
SA0, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	*Stankovski, 1986
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bruce & Heddle (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	4800	De Flora (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Rogan *et al.* (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	De Flora *et al.* (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	*Stankovski, 1986
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	4800	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	De Flora *et al*. (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	*Stankovski, 1986
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	4800	Brusick & Matheson (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	NG	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	1262	Parodi *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	NG	De Flora *et al.* (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	250	Bruce & Heddle (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	*Stankovski, 1986
SAS, *Salmonella typhimurium* TAG46, reverse mutation	–	–	15000	Bartsch *et al.* (1980)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	2000	Brusick & Matheson (1976)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	NT	120	Von Wright & Tikkanen (1980)
SCG, *Saccharomyces cerevisiae*, gene conversion	–	–	2000	Brusick & Matheson (1976)
ANF, *Aspergillus nidulans*, forward mutation	+	NT	100	Bignami *et al.* (1981)
DMM, *Drosophila melanogaster*, somatic mutation (*white/white+)*	+		150 feed	Vogel & Nivard (1993)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1200 inj	Zijlstra & Vogel (1988)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	NT	2	Sina *et al.* (1983)
URP, Unscheduled DNA synthesis, male F344 rat primary hepatocytes *in vitro*	–	NT	250	*Barfknecht (1986)
URP, Unscheduled DNA synthesis, ACI/N rat primary hepatocytes *in vitro*	–	NT	60	Mori *et al.* (1988)
UIA, Unscheduled DNA synthesis, C3HeN mouse primary hepatocytes *in vitro*	+	NT	60	Mori *et al,* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	80	Brusick & Matheson (1976)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	6	Rogers & Back (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, *ouabain resistance and cytosine arabinoside resistance in vitro*	-	NT	300	Rogers & Back (1981)
G9H Gene mutation, Chinese hamster lung V79 cells, hprt locus (metabolic activation with rat liver perfusate) *in vitro*	-	+	300	Beije *et al.* (1984)
Gene mutation, Chinese hamster ovary cells, CHO-k1BH4	EQUI	EQUI	1000	*Stankowski and Tunman (1987)
Gene mutation, Chinese hamster ovary cells, CHO-k1BH4	- / INC	- / INC	5000	*Stankowski (1988)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	–	–	5000	*SanSebastian (1986)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	(+)	20	JETOC (1997)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1950 in NMRI mouse host	–		140 po × 1	Von Wright & Tikkanen (1980)
DVA, DNA fragmentation, Swiss albino mouse lung *in vivo*	+		42 ip × 5	Parodi *et al.* (1981)
DVA, DNA fragmentation, Swiss albino mouse liver *in vivo*	+		42 ip × 5	Parodi *et al.* (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
, DNA strand breakage, COMET assay, male CD-1 mouse liver and lung *in vivo*	+		50 ip × 1	**Sasaki *et al.* (1998)
, DNA strand breakage, COMET assay, male CD-1 mouse stomach, colon, kidney, bladder, brain, bone marrow *in vivo*	-		50 ip × 1	**Sasaki *et al.* (1998)
, DNA strand breakage, COMET assay, male CD-1 mouse stomach, colon, liver, lung *in vivo*	+		50 po × 1	**Sasaki *et al.* (1998)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	–		50 ip × 1	Tyson & Mirsalis (1985)
MVM, Micronucleus test, CD1 mouse splenocytes *in vivo*	+		13.8 ip × 1	Benning *et al.* (1994)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, CD1/CR mouse bone marrow cells *in vivo*	–		83 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD1/CR mouse spermatids *in vivo*	+		83 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD1/CR mouse hepatocytes *in vivo*	+		14 ip × 2	Cliet *et al.* (1989)
MVM, Micronucleus test, (C57BL/6 × C3H/He) F_1 mouse bone-marrow *in vivo*	–		500 ip × 5	Bruce & Heddle (1979)
MVM, Micronucleus test, mouse bone marrow (BALB/c AnNCrj) *in vivo*	–		20 ip × 1	Suzuki *et al.* (1994)
DLM, Dominant lethal test, ICR/Ha Swiss mice *in vivo*	–		63 ip × 1	Epstein *et al.* (1972)
DLM, Dominant lethal test, mice *in vivo*	–		12.5 ip × 5	Brusick & Matheson (1976)
BVD, Binding (covalent) to DNA, formation of N7-methylguanine in Sprague-Dawley rat liver DNA *in vivo*	+		19 po x 1	Sagelsdorff *et al.* (1988)
SPM, Sperm abnormality test, (C57BL/6 x C3H/He)F1 mouse *in vivo*	-		500 ip x 5	Bruce & Heddle (1979)
SPM, Sperm morphology, (C57BL/6 x C3H/He)F1 mice *in vivo*	-		100 ip x 5	Wyrobek & Bruce (1975)
Colonic nuclear aberration assay in C57BL/6J mice, *in vivo*	-		100 po x 1	Wargovich *et al.* (1983)

Notes:
*: Studies marked with the asterisk were carried out with UDMH pure and protected against oxidation, in the documentation provided to EFSA.
**: in the documentation provided to EFSA.
[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, intraperitoneal; po, oral.; EQUI, equivocal, INC, inconclusive.