CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

DAMINOZIDE
(Studies with UDMH included)

Chemical Code: 000007, Tolerance # 246
SB #: 136

July 30, 1986
Revisions 7/16/87, 12/23/87, 10/27/88, 4/20/89, 11/29/89, 3/16/90, 11/16/90, 8/1/91

I. DATA GAP STATUS

Combined, rat:	No data gap, possible adverse effect (oncogenicity)
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect

DNA damage: No data gap, no adverse effect

Neurotoxicity: Not required at this time

Toxicology one-liners are attached.

In record/document number identifiers for one-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T910801

Revisions by Martz, 7/16/87; Gee, 12/23/87; Kishiyama & Davis, 10/27/88; Gee, 4/20/89; Silva, 11/29/89; Gee, 3/16/90; Aldous, 11/16/90; Kellner, 8/1/91.

Rectified with Library printout through volume 051, Record No. 097254. All relevant records available as of 7/25/91 have been examined.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

NOTE: CDFA has considered the rat chronic and oncogenicity study requirements filled as of October 1988 reviews by B. Davis. No single study was considered acceptable at that time, however Dr. Davis considered the collective data to be adequate for oncogenicity and chronic study data requirements, particularly on the strength of daminozide study 032:070327, which was faulted only on the basis of dose level justification. An earlier NCI daminozide study (005:026447) differed from current guidelines in several respects, but also provided useful information. Since the 1988 CDFA reviews, the UDMH study was completed at IRDC (043:085116). None of these studies provided definitive evidence of dose-related tumor effects. Each of the three studies used Fischer 344 rats. The NCI study (026447) provided the strongest evidence for a treatment effect on uterine adenocarcinomas. Both of the other studies provided weak additional support for a treatment effect for such tumors. In addition, both daminozide studies indicated low levels of uterine leiomyosarcomas as a possible treatment response: this was not indicated in the UDMH study. A possible increase in hepatocellular tumors was observed in females in the UDMH study only. These observations suggest marginal evidence of oncogenicity in the rat. Aldous, 11/7/90.

246-032, Parts 1-6 070327 Johnson, D.E., "Two Year Dietary Toxicity and Oncogenicity Study in Rats" (IRDC, Study No. 399-055, 8/25/88). Daminozide (Alar Technical, Lot No. M074023NB, 99.8%; UDMH content = 29 ppm) fed to Fischer 344 rats (60/sex/group) at 0, 100, 500, 5,000, and 10,000 ppm for 2 years; interim sacrifice of 10/sex/group at 1 year. **POSSIBLE ADVERSE EFFECT** (presence of comparatively uncommon uterine tumors: incidences in controls through increasing dosage groups were 0, 1, 2, 1, and 1 for uterine adenocarcinomas; and 0, 0, 0, 2, and 1 for leiomyosarcomas). A previous study (1978 NCI study at Litton Bionetics; Record 026447) also noted increases in these cell types. Chronic toxicity NOEL > 10,000 ppm (HDT); **UNACCEPTABLE**-an MTD was not achieved. Davis, 10/20/88; 1-liner incidence details added 11/7/90 by Aldous.

246-035 067727 Supplement to 070327. Contains pages missing from the initial submission - 265, 273, 481-490, 492-498, 514, 515, 645 and 653. No worksheet. Gee, 3/13/89.

246-039 072778 Supplement to 070327. Contains replacement pages for tables containing data in which one 0 ppm rat was incorrectly listed as having "malignant lymphoma, lymphocytic" but should read "mononuclear cell leukemia." No worksheet. Gee, 3/13/89.

246-017, 050911: "Two Year Dietary Toxicity and Oncogenicity Study in Rats" (12 Month Interim Report, Study #399-055) - see # 070327.

246-018, No record number; 3/11/87 letter from registrant with brief summary of new combined rat study; Contains summary of #050911 and the EPA submission date (listed as 8/15/88). Martz 6/22/87.

246-037 072994 Partial duplicate of 032:070327, above.

246-013 036932: Oser, B.L. "Chronic (2-Year) Feeding Studies With B-995 in Rats and Dogs" (FDRL, 11/15/66) Daminozide (no purity information) at 0, 300, 1000 or 3000 ppm for 2 years to 25 rats/sex/group; 37 rats/sex/control group; **UNACCEPTABLE**, **NOT UPGRADEABLE:** dose levels not justified (3000 ppm max); too few animals per group (25); no dose analysis; inadequate histopathology. **NO ADVERSE EFFECT** reported. Gee 11/26/85.

246-037 Documents submitted in support of the position that daminozide should not be considered a carcinogen until the UDMH studies are completed and reported. Comments were submitted by several consultants to the registrant and were those presented at a meeting on January 26, 1989, with CDFA. Gee, 3/10/89.

246-038 A compilation of references supporting the comments of Dr. Edward Ilgren as discussed at the January, 1989, meeting. These have not been individually reviewed for SB950 but are considered in the risk assessment process. Some reports contain record numbers while others do not. Gee, 3/10/89.

CHRONIC TOXICITY, DOG

** 036, 042 071340, 075754 "One year dietary toxicity study in dogs," (IRDC, 399-066, 11/14/88). Daminozide, technical (purity = 99%; 29 ppm UDMH and 0.06 ppm NDMA) was fed in the diet for one year at 0 (diet), 300, 3000 or 7500 ppm; 6/sex/group. No effect on any parameter measures; which included body weight, clinical observations, ophthalmology, microscopic examination. NOEL $\geq$ 7500 ppm. Previously reviewed as unacceptable (Gee, 3/10/89). Upon receipt and evaluation of information regarding dose justification, the study has been upgraded to **acceptable.** Silva, 11/15/89.

246-013 036934 Oser, B.L., "Chronic (2-Year) Feeding Studies With B-995 in Rats and Dogs" (FDRL, 11/15/66). Daminozide (no purity information) fed to 4 dogs/sex/group at levels of 0, 300, 1000 or 3000 ppm for 2 years; **UNACCEPTABLE**-needs justification of dose selection, description of a.i. purity, more details of protocol, histopathology. **NO ADVERSE EFFECT** reported. Gee 11/26/85.

ONCOGENICITY, RAT (DAMINOZIDE STUDIES)

246-005, 026447: "Bioassay of Daminozide for Possible Carcinogenicity" (NCI/Litton Bionetics, 1978) Daminozide (greater than 99%) fed in diet to Fischer 344 rats for 2 years at 5000 and 10,000 ppm; 50/sex/group in treated rats, or 20/sex/group in concurrent controls. **Possible adverse effect** (presence of the following comparatively uncommon uterine tumors): incidences in controls through increasing dosage groups were 0/19, 5/50, and 3/50 for endometrial adenocarcinomas and 0/19, 1/50, and 3/50 for leiomyosarcomas. Identification of 2 of the 4 leiomyosarcomas was later challenged on re-evaluation of the slides [see record 940, below]. Historical control incidences were 2/220 for adenocarcinoma and 0/220 for leiomyosarcoma. **UNACCEPTABLE; CANNOT BE UPGRADED**-only 20/sex in control groups; two doses (based on a subchronic study) with no evidence of MTD; higher mortality in control males may have confounded statistics. Second opinion by Martz: agree. The occurrence of rare tumor types only in treated animals indicates toxicologic significance in spite of other deficiencies.

Gee 5/6/85, with second opinion review by Martz 4/14/86, revised 5/14/86, and 11/5/90 (Aldous, no new worksheet).

246-006, 940 [in Appendix II] Supplemental to 005, 026447. "Daminozide Study Carried Out At Litton Bionetics, Inc., Rat Study," no date. Second opinion diagnoses by Dr. Stan D. Vesselinovitch, pathologist, of uterine tumors in Litton/NCI study discussed above. Dr. Vesselinovitch and the NCI pathologist differed on the diagnosis of two (of 4) leiomyosarcomas [Litton pathologist's diagnoses of "leiomyosarcoma" were changed to "sarcoma" for one low dose female, and to "neurilemoma" in one of three affected high dose females]. Diagnosis of the remaining 2, both in the high dose group, remained unchanged. Although the differences from control were not significant, a treatment-related effect still cannot be discounted, because this tumor type is rare and was found exclusively in the high dose group. Endometrial adenocarcinoma diagnosis remained unchanged, and the incidence was statistically significant. CDFA review by F. Martz, 4/14/86 (no supplemental information review worksheet prepared).

246-006, 038449: Supplemental to 005 026447. "Uniroyal Summary and Review of Daminozide and UDMH Oncogenicity Studies,"--Rat; no date; Uniroyal's summary /critique of daminozide rodent studies. CDFA review by F. Martz 5/14/86 (no supplemental information review worksheet prepared).

246-006, 000937: Supplemental to 005, 026447. "Overall Incidence of Relevant Neoplastic Lesions Identified in the Daminozide Studies," no date. Summary table. CDFA review by F. Martz, 5/14/86 (no supplemental information review worksheet prepared).

ONCOGENICITY, RAT (UDMH AND OTHER SUBSTITUTED HYDRAZINES)

****246-043 085116** Goldenthal, E. I., "Two year oncogenicity study in rats", IRDC Study 399-062, 9/28/89. Charles River Fischer-344 rats were supplied UDMH (major metabolite of Daminozide) in pH-buffered drinking water at 0, 1, 50 or 100 ppm. Seventy/sex/group were initiated on study. Of these, 20/sex/group were sacrificed at 12 months: the remainder were maintained for 2 years. Purity of UDMH was not specified, however solutions were satisfactorily assayed against a commercial standard. NOEL = 1 ppm (decreased water consumption in females, possibly due to smell or taste aversion to test article). A practical

level for which no apparently meaningful toxicity is observed would be 50 ppm in males and females. At 100 ppm there were modest, but consistent body weight decrements in both sexes, in conjunction with reduced water consumption (especially in females). **Possible adverse effects** were noted: a modest increase in incidence of hepatocellular tumors in 50 and 100 ppm females, also the presence of two high dose females with comparatively rare uterine adenocarcinomas, provide marginal evidence of treatment effects. Aldous, 11/16/90.

246-049 073813 A copy of R. A. Squire’s analysis of hepatocellular tumors and related lesions in rat study 043:085116, above. The same analysis is found in part 6 of the final report in Vol. 043.

ONCOGENICITY, MOUSE

****246-031, Parts 1-5 070326** Johnson, D.E., "Two Year Dietary Oncogenicity Study in Mice" (IRDC, Study No. 399-054, 8/29/88). Daminozide (Alar Technical, Lot No. M074023NB, 99.8%: UDMH content = 29 ppm) fed to CD-1 mice (50 mice/sex/group) at 0, 300, 3,000, 6,000, and 10,000 ppm for 2 years. This study did not demonstrate an MTD, however dose levels are justifiable on the basis of earlier studies (see 005:026448). Brown pigmentation in livers of 6000 and 10000 ppm males suggested a modest treatment effect, with a NOEL of 3000 ppm for males. Apparent NOEL for non-neoplasia effects in females was 10000 ppm. A **"possible adverse effect"** was equivocal evidence of a high dose effect on vascular tumor (hemangioma plus hemangiosarcoma) incidences in livers of 10000 ppm males and in uteri of 10000 ppm females. Evidence of a pulmonary tumor effect was insufficient to warrant a "possible adverse effects" call. (The 1988 CDFA review had considered the pulmonary tumors to indicate a "possible adverse effect"). **Acceptable**. Davis, 10/20/88; Aldous, 11/7/90. [Note: high doses of UDMH predictably elicit vascular tumors and pulmonary tumors. See individual 1-liners in the "substituted hydrazines" section, below].

246-037 072993 Partial duplicate of 031:070326, above.

019, 057774: "Two Year Dietary Oncogenicity Study in Mice," IRDC, 10/30/86; one year interim report cited in the letter of 11/24/86.

029, 065260: Interim pathology report for Record 070326. Davis 10/7/88.

018, No record number: Letter from registrant dated 3/11/87 with brief summary of new studies in progress including the repeat mouse study [see 070326] Martz 6/22/87.

017, No record number; Cover letter from registrant dated 11/24/86: similar information to that given above in 018. Martz 7/16/87.

246-005, 026448: "Bioassay of Daminozide for Possible Carcinogenicity"--Mouse, NCI/Litton Bionetics, 1978; daminozide (greater than 99% purity) to B6C3F1 mice at 5000 and 10,000 ppm in feed, 2 years. 50/sex/group, 20/sex/group for controls; **ONCOGENIC EFFECT** - hepatocellular carcinomas in males, dose-related trend with significance at high dose level. Lung tumors (alveolar/bronchiolar adenomas and carcinomas) in females, not significant by Fisher's. Note that lung tumors were also reported in Swiss mice by Eppley (see below, # 023502) and in CD-1 mice by IRDC (see above, # 070326). **UNACCEPTABLE & CANNOT BE UPGRADED**: dose selection (no evidence of toxicity), too few controls, two doses only. Gee 5/6/85.

246-038 072541 Duplicate of 026447 and 026448 (above).

246-006, 038450: Supplemental to 005, 026448. "Daminozide Study Carried Out At Litton Bionetics, Inc., Mouse Study," no date, Second opinion review by Dr. Stan D. Vesselinovitch, pathologist, of selected liver tumors in male and female mice of the Litton/NCI study discussed above. There were no substantial differences in tumor diagnoses between Dr. Vesselinovitch and the NCI pathologist. Martz 5/14/86 (no supplemental information review worksheet prepared).

246-006, 031139 & 038451: Supplemental to 005, 023502 & 026448. "Overall Incidence of Relevant Neoplastic Lesions Identified in the Daminozide Studies" in mice, no date. Summary comparison table. Martz, 5/14/86 (no supplemental information review worksheet prepared).

246-006, 945 & 946: Supplemental to 005, 023502 & 026448. "Uniroyal Summary and Review of Daminozide and UDMH Oncogenicity Studies,"--Mice; no date; Uniroyal's summary/critique of daminozide rodent studies. Martz, 5/14/86 (no supplemental information review worksheet prepared).

246-005, 023502: Toth, B., Wallcave, L., Patil, K., Schmeltz, I., and Hoffmann, D., "Induction of Tumors in Mice With the Herbicide Succinic Acid 2,2-Dimethylhydrazide," Cancer Research 37, 3497-3500, from Eppley Inst., 10/77; Oral dosing in drinking water at 2% concentration, equivalent to 3000 mg/kg/day, in Swiss mice, 100/sex in controls and 50/sex in treatment group. Contained 0.002% UDMH (40 ppm), known to be tumorigenic in mouse lung (see #23503 below), equivalent to 6 mg/kg/day; **ONCOGENICITY** in lungs (primarily adenomas, with some adenocarcinomas), blood vessels (particularly angiosarcomas, with some angiomas: these were most abundant in the liver), and kidney (adenomas). Lung tumors probably due to UDMH contaminant rather than test article itself. Treatment relatedness of kidney tumors is subject to question (see below). Nevertheless, incidences of vascular tumors merit attention in spite of scientific shortcomings. Study is **UNACCEPTABLE** for regulatory purposes due to multiple deficiencies, such as grossly exceeding the MTD so that survival was severely reduced: study **CANNOT BE UPGRADED**. Gee 5/6/85, with second opinion Martz 5/14/86.

246-038 072765 Duplicate of 005:023502, above.

246-006, 000941 & 000942: Supplemental to 005, 023502 above. "Daminozide Study Carried Out at Litton Bionetics, Inc.," mice, no date. Second opinion diagnoses by Dr. Stan D. Vesselinovitch, pathologist, of tumors in Eppley study discussed above. Dr. Vesselinovitch and the Eppley pathologist differed on numerous diagnoses such that the incidence of vascular tumors in liver was reduced (but still significant), and kidney tumor incidence was reduced and no longer significant. Lung tumor incidence remained unchanged from original diagnoses.

The consultant audited the data files and found substantial evidence of poor study conduct. Consequently, the results are subject to question. Nevertheless, the significantly elevated incidences of lung and vascular tumors can not be discounted in spite of this study's deficiencies. Martz 5/14/86 (no supplemental information review worksheet prepared).

246-006, 000938: Supplemental to 005, 023502; "Development of Blood Vessel Tumors in Swiss Mice (Eppley Subline) as Reported by Bela Toth," no date. Incidence of vascular tumors in male and female controls from 18 references. Martz 5/14/86 (no supplemental information review worksheet prepared).

ONCOGENICITY, MOUSE (UDMH AND OTHER SUBSTITUTED HYDRAZINES)

NOTE: Taken together, the two IRDC studies below (Volumes 44 and 48) show clear potential for UDMH to elicit bronchiolar/alveolar tumors as well as vascular tumors (particularly hemangiosarcomas, commonly observed in liver) in both sexes at or above 40 ppm. In addition, the lung tumors were observed in 20 ppm females. Minimum doses associated with tumor development approximated the MTDs in respective sexes. Although the "possible adverse effect" of UDMH cannot be ignored, the findings below should be considered in the context of possible excessive toxicity. Aldous, 10/22/90.

****246-048 090438** Goldenthal, Edwin I., "Two year oncogenicity study in mice", IRDC, 1/31/90. CD-1 mice were supplied UDMH (major metabolite of Daminozide) in pH-buffered drinking water at 0, 40, or 80 ppm. Out of 90/sex/group originally assigned, 20/sex/group were sacrificed at 8 months and an additional 20/sex/group at 12 months: the remainder were maintained for 2 years. Purity of UDMH was not specified. **Possible adverse effects** were noted: vascular tumors (particularly hemangiosarcomas in liver) and alveolar/bronchiolar tumors (primarily adenomas) were each elevated in both sexes at both dosages. **The high doses (80 ppm) in both sexes clearly exceeded the MTD**, based on survival criteria. Reduced survival at the 40 ppm dose in females may also have represented a treatment effect, hence tumor effects must be interpreted in the context of high toxicity. Liver toxicity (and/or adaptive responses) was demonstrated

by hypertrophy, necrosis, and brown pigmentation in both sexes at both dosages. In addition, an inflammatory response was evident in males at both doses. Serum chemistry findings of elevated alanine aminotransferase (SGPT), and sorbitol dehydrogenase at both dose levels in both sexes substantiate other indications of a liver response. Liver pigment analyses indicated possible hemolysis, cholestasis, and tissue damage and repair: these findings were more prominent in males than in females. Extramedullary hematopoiesis (especially in spleen) was consistent with hemolysis. No unique lung toxicity appeared to be related to lung tumors. This study should be considered along with the related low dose study, 044:085117 (IRDC study no. 399-063: completed 9/28/89). **Acceptable** in conjunction with study in Vol. 44. Aldous, 11/16/90.

246-045 085118 Preliminary pathology report for 048:090438, above. This is preceded by a letter from R. Cardona, who provided evidences that the above study had employed excessively high dose levels, and thus should not be used for risk assessment. (No CDFA worksheet). Aldous, 11/2/90.

246-033 070816 12--month interim report for 048:090438, above.

****246-044 085117** Goldenthal, E. I., "Two year oncogenicity study in mice", IRDC Study 399-063, 9/28/89. CD-1 mice were supplied UDMH (major metabolite of Daminozide) in pH-buffered drinking water at 0, 1, 5, and 10 ppm (males) or 20 ppm (females). Ninety/sex/group were initiated on study. Of these, 20/sex/group were sacrificed at 8 months and an additional 20/sex/group at 12 months: the remainder were maintained for 2 years. Purity of UDMH was not specified. **A possible adverse effect** was noted: increased incidence of alveolar bronchiolar adenomas and carcinomas in 20 ppm females. NOEL = 1 ppm in males (pigment accumulation in liver) and females (single cell necrosis in liver). It appeared that survival was reduced in 20 ppm females during months 19-21: this was the main indication that the MTD may have been exceeded. There were no (non-neoplasia) treatment effects in lungs corresponding to observed tumors. Liver findings at 10 or 20 ppm included hypertrophy, hyperplasia, and pigmentation: none of these changes were marked in degree or incidence. Acceptable (study should be considered in light of the higher dose study, 048:090438). Aldous, 11/16/90.

246-038 072545 Toth, B., "Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors." Int. J. Cancer:9:109-118 (1972). C3H mice were given solutions of above materials in drinking water over their entire lifetimes. Dose levels were 0.001% for hydrazine, 0.01% for methylhydrazine and 0.01% for methylhydrazine sulfate. Author indicated that hydrazine and methylhydrazine sulfate as administered increased incidence of lung tumors (adenomas and adenocarcinomas), and that latency of such tumors was shortened for methylhydrazine (which markedly increased mortality at the 0.01% level administered). Study is unacceptable (very limited protocol, only one dose level, insufficient information for independent assessment), but provides limited information related to neoplasia potential of UDMH. Aldous (no CDFA worksheet), 11/1/90.

246-038 072548 Toth, B., "The large bowel carcinogenic effects of hydrazines and related compounds occurring in nature and in the environment". Cancer 40:2427-2431 (1977). A 0.1% level of 1,1-dimethylhydrazine [= UDMH] in drinking water administered over the lifetime to Syrian golden hamsters was stated to have reduced survival and to have elicited tumors of the cecum (including polypoid adenomas and adenocarcinomas). Study is unacceptable (very limited protocol, only one dose level, insufficient information for independent assessment), but provides limited information related to neoplasia potential of UDMH. Aldous (no CDFA worksheet), 11/1/90.

246-038 072764 Toth, B. and Wilson, R.B., "Blood vessel tumorigenesis by 1,2-dimethylhydrazine dihydrochloride (symmetrical)". Amer. J. Pathol. 64:585-600 (1971). Administration of 0.001% 1,2-dimethylhydrazine dihydrochloride (symmetrical) in drinking water of randomly bred Swiss mice reduced survival. There was a remarkable increase in incidence of angiosarcomas in both sexes, for which background incidence was very low, with apparent reduction of latency. There was also an increase in comparatively common lung tumors (primarily adenomas), with a corresponding decrease in latency. Study is unacceptable (test article is not the isomer of concern, very limited protocol, only one dose level, insufficient information for independent assessment), but provides limited information related to neoplasia potential of the related compound, 1,1-dimethylhydrazine (UDMH). Aldous (no CDFA worksheet), 11/2/90.

246-005, 023503: "Carcinogenicity of Hydrazine and 1,1-Dimethylhydrazine for Mouse Lung," (l967, publ. in Nature, from Chester Beatty). 1,1-dimethylhydrazine (UDMH, daminozide contaminant and metabolite), 0.5 mg/mouse, presumed equivalent to about 20 mg/kg, by gavage to female Swiss mice 5 days a week for 40 weeks. Dose >>>MTD, based on mortality (meaning??); ONCOGENICITY - Significant increase in lung tumors, alveolo/bronchiogenic adenomas or adenocarcinomas, at multiple sites. Study **UNACCEPTABLE** & **CANNOT BE UPGRADED** for regulatory purposes, but sheds some light on the tumorigenic properties of daminozide contaminated with UDMH reported by Eppley. Gee 5/6/85 with second opinion review by Martz 5/14/86.

006, 000939: "1,1-Dimethyl Hydrazine Study Carried Out at Eppley Cancer Institute" 1973 publication; 1,1-Dimethylhydrazine (UDMH -no purity information), 0.01% in drinking water until 100% mortality; Report consists of a second opinion diagnosis of hepatocellular tumors by pathologist Dr. Stan D. Vesselinovitch (see letter dated 4-10-84 in same volume) of slides previously diagnosed by principal investigator Bela Toth. Many liver vascular tumors were rediagnosed as being non-neoplastic hepatotoxic lesions. Discrepancy is moot because new studies with daminozide and UDMH are in progress, see -018 above. Martz 7/15/87.

REPRODUCTION, RAT

** 023, 059837: "Two Generation Reproduction Study with ALAR in Rats (One Litter per Generation)" (Hazleton, HLA Study No. 6111-102, 5/26/87) Alar (Lot No. MO 55030, 99% pure daminozide) fed to 25 Sprague Dawley rats/sex/dose at 0, 100, 1000 or 10,000 ppm in diet in F_0 and F_1 generations; F_1 and F_2 generations exposed in utero and via lactation; study terminated when F_2 reached 21 days of age; **NO ADVERSE EFFECT;** initially reviewed as not upgradeable with insufficient dose selection rationale - no evidence of toxicity (in any parameter measured) in F_0 and F_1 rats fed the highest dose. NOEL $\geq$ 10,000 ppm (HDT). Becker 10/28/87, Kishiyama & Davis 10/11/88. Document 246-047, Record 088035, contains a rebuttal and a review by FAO/WHO panel accepting the study with a NOAEL = 1000 ppm. Re-consideration of the total data base and the use of a high dose of 10,000 ppm, the study is **ACCEPTABLE.** Gee, 3/16/90.

246-037 072998 Partial duplicate of 023:059837, above.

030, 066544: "Data Evaluation Record. Alar. Two-Generation Reproduction Study in Rats" Contract review by Dynamac Corporation for EPA. EPA set parental toxicity NOEL = 1,000 ppm & LEL = 10,000 ppm based on reduced body weight in F1 parental males; reproductive/developmental toxicity NOEL = 100 ppm & LEL = 1,000 ppm based on delayed mating in treated males. EPA accepted the study as Core-Minimum. CDFA review did not alter previous review. Kishiyama & Davis 10/11/88.

013, 036933: "Chronic (2-Year) Feeding Studies With B-995 in Rats and Dogs -Reproduction and Lactation," FDRL, 11/15/66; daminozide (no purity information) fed to 20 rats/sex at 0 or 300 ppm in F_0 generation and to 10/sex/group for F_1 and F_2 parental generations, F_0 part of chronic combined study (record #036932), **NO ADVERSE EFFECT** reported, **INCOMPLETE** (missing individual data), **UNACCEPTABLE, CANNOT BE UPGRADED** (too few animals, single dose (300 ppm) with no justification, no analysis of diet). Gee 11/26/85.

TERATOLOGY, RAT

** 246-004, 000950: "Teratologic Evaluation of Alar Technical in Sprague-Dawley Rats" FDRL, 3/6/79; daminozide (Alar technical, no purity information) at 0, 85, 390 and 1800 mg/kg by oral gavage days 6-15 of gestation; Positive control was aspirin at 1800 mg/kg; **NO SIGNIFICANT ADVERSE EFFECTS** in absence of maternal toxicity; NOEL = 390 mg/kg for developmental and maternal effects. **ACCEPTABLE.** Gee 5/3/85 with second opinion by Parker on 3/26/86.

246-014, 036937: "Teratogenic Study with ALAR in Albino Rats," IBT, 11/28/72; **Invalid** IBT study, IBT No. B1708, Dose levels were 250, 500 mg/kg; days 6-15 to rats. Gee 5-3-85

246-005, 023505: "Teratologic Assessment of Maleic Hydrazide and Daminozide, and Formulations of Ethoxyquin, Thiabendazole and Naled in Rats," [J. Env. Sci. Health B14(6), 563-577, 1979], Daminozide (99%) at 0, 200, 600 or 1000 mg/kg days 6-15 of gestation by oral gavage; **NO ADVERSE EFFECT** reported; **INCOMPLETE** (No individual data, no analysis of dosing solution); **UNACCEPTABLE** (need justification of dose-dose levels not high enough, justification & description of use of water as a vehicle) Could be upgraded. Gee 5/6/85.

TERATOLOGY, RABBIT

** 246-013, 036935: "Teratology Study in Rabbits (Alar)" IRDC, 8/7/85; daminozide, 99% pure, by oral gavage in CMC at 0, 50, 150 or 300 mg/kg/day to 16 rabbits/ group, days 7-19 of

gestation; maternal effects were 1 death and reduced weight gain at 300 mg/kg; **NO ADVERSE DEVELOPMENTAL EFFECTS**, developmental NOEL > 300 mg/kg. **ACCEPTABLE** with minor variances. Gee 11/26/85.

246-007, 000957: "Toxicological Studies on Succinic Acid 2,2-dimethyl Hydrazide (ALAR)--Somer's Test"; Brown Biological Laboratories, no date; daminozide (no purity information) at 0, 42 or 126 mg/kg by oral gavage days 7-16 of gestation, half of animals sacrificed day 28, remainder allowed to go to term; **INSUFFICIENT INFORMATION FOR ADVERSE EFFECTS ASSESSMENT; INCOMPLETE** (missing some individual data). **UNACCEPTABLE AND CANNOT BE UPGRADED** (no toxicity at high dose). Gee 5/3/85.

GENE MUTATION

007, 000951: "Mutagenicity Evaluation of B995 (ALAR)" Litton Bionetics, 5/18/77; Ames test with Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 and Saccharomyces cerevisiae strain D4; Daminozide (no purity information) at 0.1, 1, 10, 100, or 500 ug/plate with and without activation, No effects reported, but has **insufficient information for assessment; UNACCEPTABLE, CANNOT BE UPGRADED** (too few plates, dose level too low, and no repeat test for confirmation). Gee 5/3/85

006, 000935: "P7642: Investigation of Mutagenic Activity in the TK+/- Mouse Lymphoma Cell Mutation Assay;" Life Sci. Res., 9/82); Mouse lymphoma L5178Y cells; Daminozide technical (no purity information) at 0, 1500, 2000, 2333.3, 2666.7 or 3000 ug/l with and without activation; **NO ADVERSE EFFECT** indicated; originally accepted on 5/7/85 (J.R. Gee), but downgraded to **UNACCEPTABLE** on basis of no repeat trial. Gee 5/7/85 and 7/2/87.

006, 000943: "Uniroyal Summary and Review of Daminozide and UDMH Oncogenicity Studies"--Mutagenicity Studies, no date; Brief literature review of daminozide and UDMH mutagenicity studies, Cites **ADVERSE EFFECT** in a mouse lymphoma assay for UDMH and states that UDMH was

inactive or marginally active in several Ames (Salmonella) assays, Very Brief Summary, **SUPPLEMENTAL**.

** 246-051 097254 "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay", (Richard H.C. San, Ph.D. and J. Blair Shelton, B.S., Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD, Report # T9292.501014, 3/21/91). Daminozide, lot #806M006NB, 99.8% purity, was tested in the reversion assay with Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation by Aroclor 1254 stimulated rat liver S-9 fraction with 3 plates/strain/dose in 2 experiments at concentrations of 0 (DMSO control), 667, 1000, 3333, 6667 and 10000 ug/plate. No adverse effects were noted (no increase in the number of revertant colonies). **Acceptable.** (Green, Kellner and Gee, 7/24/91).

246-050 096353 Partial duplicate of 051:097254, above.

CHROMOSOME EFFECTS

246-007, 000953: "Dominant Lethal Assay of Alar in the Male Mouse" Huntingdon Res. Ctr., 6/22/73; daminozide (no purity information) at 0, 10, 300 or 10,000 ppm to 20 CFLP mice/group (3 doses based on preliminary study); MTD not achieved; **NO ADVERSE EFFECT** indicated; **INCOMPLETE** (no individual data); **UNACCEPTABLE, CANNOT BE UPGRADED** (no concurrent positive control, too few females, only four weeks of testing). Gee 5/3/85.

** 246-051 097253, "Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells", (Donald L. Putman, Ph.D. and Marcia J. Morris, B.A., Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD., Report # T9292.337, 2/20/91). Daminozide, lot #806M006 NB, 99.8% purity, was tested for chromosomal aberrations in Chinese Hamster ovary cells ($CHO\text{-}K_1$) with and without metabolic activation by Aroclor 1254 stimulated rat liver S-9 fraction with 2 cultures/dose at levels of 0 (DMSO), 250, 500, 1000, and 2000 ug/ml. No adverse effects were noted (no increase in the proportion of aberrant metaphases). **Acceptable.** (Green, Kellner and Gee, 7/25/91).

246-050 096354 partial duplicate of 246-051:097253, above.

DNA DAMAGE

** 246-006, 000934: "P7642: Assessment of Its Ability to Induce Primary DNA Damage in Strains of Εσχηεριχηια χολι" Life Sci. Res., 7/16/82, E. χολι with and without activation; daminozide (no purity information) at 250, 1000, 2500 and 10,000 ug/ml; **NO EVIDENCE OF DNA DAMAGE; COMPLETE, ACCEPTABLE**. Gee 5/7/85.

** 246-006, 000936: "P7642: Assessment of its Ability to Induce Genetic Damage in Σαχχηαρομψχεσ χερεϖισιαε" Life Sci. Res., 1/21/83, Σαχχηαρομψχεσ χερεϖισιαε strain D6; Daminozide (no purity information) at 1-2000 ug/ml (11 concentrations) with and without activation; **NO EVIDENCE OF ADVERSE EFFECT** (mitotic crossing over); **COMPLETE, ACCEPTABLE**. Gee 5/6/85.

NEUROTOXICITY

Not required at this time.

GENOTOXICITY STUDIES WITH UDMH OR RELATED HYDRAZINES

GENE MUTATION

019 057778 "Ames/SALMONELLA Plate Incorporation Assay" (Pharmakon Research International, PH 301-UN-005-086, 12/9/86) Unsymmetrical dimethyl hydrazine (UDMH), lot JS-34, E732-086, no purity stated; tested with Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 at 0, 25, 83.3, 250, 833, 2500 and 5000 ug/plate in triplicate, one trial with cytotoxicity (reduced colonies or change in background lawn) at 5000 ug/plate; **NO EVIDENCE FOR AN INCREASE IN REVERSION RATE**; conducted in accordance with EPA guidelines as modified in May, 1987. **SUPPLEMENTAL DATA** (derivative of daminozide). Gee 12/23/87.

019 057777 "CHO/HPRT: Mammalian Cell Forward Gene Mutation Assay" (Pharmakon Research International, 1/16/87, PH 314-UN-001-086) Unsymmetrical dimethyl hydrazine (UDMH), lot JS-34, E732-086), purity not stated; tested with Chinese hamster ovary cells for gene mutation of the HGPRT locus to thioguanine resistance at 0, 50, 100, 250, 500, 750 or 1000 ug/ml with no cytotoxicity, the high concentration limited by the solvent (HCl); no consistent effect on mutation frequency although some samples were statistically significant - **RESULTS CONSIDERED EQUIVOCAL; SUPPLEMENTAL DATA** (derivative of daminozide). Gee 12/23/87.

034 070968 "Unsymmetrical Dimethylhydrazide (UDMH) CHO/HPRT Mammalian Cell Forward Gene Mutation Assay" (Pharmakon Research International, Inc., Laboratory Project ID: PH 314-UN-001-88, 9/12/88) Duplicate cultures of Chinese Hamster Ovary cells (CHO-K1-BH4) were exposed to UDMH = 1,1-Dimethylhydrazine at 0, 50, 100, 250, 500, 1000, 2500, 3750, and 5000 ug/ml ± S9 in one assay and at 0, 50, 100, 250, 500, 1000, 1500, 2000, 2500, 3000, 3500, 3750, 4000, 4500, and 5000 ug/ml ± S9 in a second assay; **NO ADVERSE EFFECT**-A 3-fold increase in mutation rate in the first assay at 3750 ug/ml + S9 was not confirmed in the second assay; cytotoxicity was demonstrated; **SUPPLEMENTAL STUDY;** Davis 10/26/88.

CHROMOSOME EFFECTS

019 057775 "In vitro Chromosome Aberration Analysis in Chinese Hamster Ovary (CHO) Cells" (Pharmakon Research International, 12/6/86, PH 320-UN-002-86), UDMH (25,000 ppm in stock - no solvent given); tested in CHO cells stated in the report to be 0, 500, 1500 or 5000 ug/ml, but from raw data, high concentration is 500 ug/ml, 5-hour exposure (6 hours according to raw data) with and without Aroclor-induced rat liver followed by 18 hours in BrdUrd; duplicate cultures, scored 2 x 50 cells per concentration; solvent was 0.25 N HCl at 1 or 2% final concentration (unclear which from report); **NO INCREASE IN ABERRATIONS** reported; **SUPPLEMENTAL DATA** (derivative of daminozide). Gee 12/22/87. EPA: **UNACCEPTABLE**. See Pesticide and Toxic Chemical News, June 17, 1987.

DNA DAMAGE

019 057776 "Rat Hepatocyte Primary Culture/DNA Repair Test" (Pharmakon Research International, Inc., PH 311-UN-0001-86, 12/6/86) Unsymmetrical dimethyl hydrazine (UDMH), lot JS-34, E732-086, 25 mg/ml; tested with rat hepatocytes at 0, 8.3, 25, 83 and 250 ug/ml, 18 - 20 hours; scored 150 cells by autoradiography per concentration; 0.25 N HCl, the vehicle; **NO INCREASE IN UNSCHEDULED DNA SYNTHESIS** reported; **SUPPLEMENTAL DATA** (derivative of daminozide). Gee 12/23/87.