CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

2-16-89

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: California Department of Food and Agriculture - EPA Toxicology Review for Amitraz

TOX Chem No. 374A

FROM: Ray Landolt 2/16/89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and Antimicrobial Support
Health Effects Division (TS-769C)

TO: William Burnam, Acting Director
Health Effects Division (TS-769C)

THRU: Mike Ioannou, Acting Section Head JM Ioannou 2-16-89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and Antimicrobial Support
Health Effects Division (TS-769C)

The following responses are provided for each specific deficiency identified by the Medical Toxicology Branch of the California Department of Food and Agriculture (CDFA):

Study Type: Dominant Lethal Assay of Amitraz in the Female Mouse
Report No. TX 77020 March 25,1977, Batch No. DJ 2703

1. Deficiency: "no purity of test material"

   EPA Response: The purity of Batch No. DJ 2703 was identified in Report No.TX 78037 as non-stabilized Amitraz of 97.1 %.

2. Deficiency: "no analysis of dosing solutions"

   EPA Response: The testing guidelines do not specifically ask for this, however, this study was performed before the November 29, 1983 Good Laboratory Practice Standards and the September 27, 1985 Genetic Toxicity Guidelines were promulgated.

/

3. Deficiency : "inadequate number of females per group"

   EPA Response: Agree. The number of females needs to be of such a number to provide appropriate sensitivity of detection and power of significance. For the male dominant lethal assay, as an example, between 30-50 pregnant females are necessary per mating interval.

4. Deficiency: "dosing of females instead of males"

   EPA Response: Usually males are used as the dosed animal, but this does not preclude dosing females. However, there are problems with dosing females and the guidelines are written for dosing males.

5. Deficiency: "no justification of doses with no toxicity reported"

   EPA Response: Agree

CONCLUSION : Agree with CDFA, this is an unacceptable study. Also, no concurrent positive controls were used nor were there results reported of positive controls examined within one year period of of this study. It is suggested that with minimal or no toxicity in this assay and a possible effect on post-implantation loss at the 19-23 day post-mating interval at 50 mg/kg dose, this assay should be performed at higher doses.

STUDY TYPE : Dominant Lethal Assay of Amitraz in the Male Mouse
Report No.TX 77021, March 29,1977, Batch No.DJ 2703

1. Deficiency: "no purity of test material"

   EPA Response: The purity of Batch No.DJ 2703 was identified in Report No.TX 78037 as non-stabilized Amitraz of 97.1%.

2. Deficiency: "no justification of dose selection with minimal signs of toxicity".

   EPA Response: Agree

3. Deficiency: "no analysis of dosing solutions"

EPA Response: The testing guidelines do not specifically ask for this, however, this study was performed before the November 29, 1983 Good Laboratory Practice Standards and the September 27, 1985 Genetic Toxicity Guidelines were promulgated.

Conclusions: Agree with CDFA, this is an unacceptable study. Additional deficiencies include: mating did not cover entire spermatogenesis cycle, usually 8 weeks; no concurrent positive controls were used nor were there results reported of positive controls examined within one year period of this study; number of pregnant females/mating interval were not large enough, the guidelines suggest 30-50 per group (in this study 20 males/ group were dosed and mated 1:1 per mating interval).

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

AMITRAZ

SB 950-213, Tolerance # 00287

August 5, 1986
(Revised 7/27/87)
Revised September 23, 1988

I. DATA GAP STATUS

Chronic rat:	See "Combined rat" below
Chronic dog:	No data gap, no adverse effects
Combined rat:	No data gap, no adverse effects
Oncogenicity mouse:	No data gap, possible adverse effects
Reproduction rat:	No data gap, possible adverse effects
Teratology rat:	No data gap, no adverse effect
Teratology rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effects with metabolite
Chromosomal aberration:	Data gap, inadequate studies on file, no adverse effects indicated
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time

Note, Toxicology one-liners are attached

** indicates acceptable study
Bold face indicates possible adverse effect
File name: T880921
Revised 7/27/87 by F. Martz and 9/21/88 by J. Gee

II. TOXICOLOGY ONE-LINERS AND DISCUSSION

CHRONIC RAT
(See "Combined rat" below)

CHRONIC DOG

** -012, 984555 "BTS 27 419: Two-Year Oral Toxicity Study in Dogs." (Boots, 9/73, TX 73075) Amitraz (purity given as 97.8 - 99.8% - see 051314 in 054), given orally in gelatin capsules to beagle dogs for two years at 0, 0.1, 0.25 and 1.0 mg/Kg/day, 4/sex/group; slight CNS depression at high dose; NOEL not apparent; initially reviewed as unacceptable (no description of test article, no age at start, no MTD), and not upgradable. A. Apostolou, 5/15/85.
-054, Rebuttal letter dated of 12/5/86 and record #51314: test article characterization, age of dogs, and dose level justification. No change in status - lack of ophthalmoscopic examinations (not noted in original review) is an uncorrectable major deficiency. Study remains unacceptable and considered not upgradable. (No supplemental information worksheet prepared) F. Martz, 7/24/87.
-065 059539 Supplement to 984555. Results of ophthalmoscopy and "macroscopic clinical ophthalmic examinations" at weeks 52, 78 and 103 for all animals. Compiled from raw data and submitted as addendum number 2 for study TX 73035. Addendum dated 1/28/88. Original report did not indicate ophthalmological exams were performed. Collective data upgrade the study to acceptable status. Gee, 9/21/88.
EPA one-liner: Systemic NOEL = 0.25 mg/Kg; no grade given. [Systemic NOEL presumably based on "CNS depression" days 1 and 2. This could be mitigated by an altered dosing regime the first several days. A 90-day study at 4 mg/Kg/day showed "minimal" signs. J. R. Gee, 7/29/86]

-049 & 051, 036397 & 044438 Exact duplicates of 984555 above.

-044, 002821 Summary of 984555 above.

-012, 984551 & 984553 Interim reports for 984555 above.

Summary: The collective data in the initial report and the addenda plus the rebuttal provide adequate data to determine the chronic effect(s) of amitraz in a non-rodent species, fulfilling the data requirement. The October, 1987 document from EPA, "Guidance for the Reregistration of Pesticide Products Containing Amitraz as an Active Ingredient", indictes that EPA considered the study as acceptable. Gee, 9/21/88.

COMBINED RAT

** -049, 036396 "BTS 27 419: Carcinogenicity and Long-Term Toxicity Study in Rats." (Boots, 11/73, TX73043) Amitraz (97.8 - 99.8% - # 051314 for purity of lots 2093 DH and 2099 DH); fed in the diet for 104 weeks to Wistar rats at 0 (diet), 15, 50 and 200 ppm; 40/sex/group; nominal systemic NOEL = 50 ppm (decreased body weight gain, decreased food intake in males); no

major adverse effect; initially reviewed as unacceptable (no analysis of dosing material, no ophthalmology exams, incomplete serum chemistry, test article not adequately characterized, no convincing evidence that MTD achieved), probably not upgradable. Marginal effects on body weight gain, food consumption, as well as behavioral effects, were noted by FM; nonetheless, this reviewer considers these responses insufficient justification of the high dose level. F. Martz, 1/30/86.

-054, Rebuttal letter dated 12/5/86 and record nos. 51321, 51315, 51316, 51309, 51317: test article characterization, retrospective feed stability analysis, retrospective feed content analysis, three month gavage rat study report and three month feed rat study report, respectively. #51321 shows amitraz purities of 97.8% and 99.8% for the lots of material used; #51315 shows 30% loss of activity in one week; #51316 shows that blends can generally be prepared correctly; #51309 and #51317 are used to justify dose level selection. Supplemental information did not change study status because of lack of ophthalmoscopic examinations. Study remained unacceptable and not upgradable. (No supplemental information worksheet prepared) F. Martz, 7/24/87.

-065 No record number. Statement from registrant that, since eye exams were done in the dog study and histopathological data are available for the rat, mouse and dog, the rat study should be acceptable. Although the lack of ophthalmological exam in the rats is a major deviation from current guidelines, since data are available in the dog study, CDFA will agree with the statement of the registrant. Considering the collective data in the subchronic studies (-054, 051309 and 051317) for dose selection and clinical chemistry/hematology/urinalysis data, CDFA now considers that there are sufficient data in the rat to address the lack of an adverse effect to doses of 200 ppm - approximately 12 mg/kg/day in males and 10 mg/kg/day in females. Gee, 9/22/88.

EPA one-liner: Systemic NOEL = 50 ppm; oncogenic NOEL>200 ppm; no grade given. [The reregistration standard issued by EPA dated October, 1987, indicated that the study was adequate.]

-010, 984559 Exact duplicate of 036396 above. [Reviewed 5/14/85 by A. Apostolou with similar conclusions]

-044, 002822 Summary of 36396 above.

-010, 984549 One year interim report for 036396 above.

ONCOGENICITY MOUSE

** -036 - 40, 001090 "Amitraz: 104 Week Tumorigenicity Study in Mice - Final Report." (Huntingdon Research Centre, 9/83) Amitraz (lot 34732Y) fed in the diet to B6C3F1 mice for 104 weeks at 0, 25, 100 and 400 ppm; 100/sex in controls, 75/sex/group in groups exposed to test article; hyperplastic nodules in liver and heptocellular carcinoma in females at high dose; decreased body weight gain at 100 and 400 ppm; decreased food consumption early in study especially at high dose; lower myeloid/erythroid ratio at 400 ppm in males and at 100 and 400 ppm in females; focal hyperkeratosis of the forestomach at terminal sacrifice in all of treated groups of males (see below); systemic NOEL < 25 ppm, tentative onco NOEL = 100 ppm; ACCEPTABLE. A. Apostolou, 5/15/85, second opinion by J. R. Gee, 8/5/86.

Finding	Control		25		100		400	
	M	F	M	F	M	F	M	F
Focal hyperkeratosis of forestomach	20/80	26/80	34/65	29/61	34/60	33/62	45/55	16/56
Hepatocellular adenoma/carcinoma	9/80	5/80	7/65	1/61	5/60	3/62	6/55	24/56

EPA one-liner: Systemic NOEL < 25 ppm; onco NOEL deferred for risk assessment; Core grade--minimum.

-037 & 040, 001091-001094 Supplements (individual data) to 001090 above.

-048, 002813 & 006474 Supplements (comments on onco effects) to 001090 above.

-048, 036709 Summary of 001090 above.

-011, 984561 "BTS 27 419: 80-Week Carcinogenicity Study in Mice - Final Report." (Boots, 5/76) Amitraz (purity not stated) fed in the diet to CFLP mice for 80 weeks at 0, 25, 100 and 400 ppm; 50/sex/group; lymphoreticular tumors in females at 400 ppm; systemic NOEL=100 ppm (body weight), onco NOEL = 100 ppm; UNACCEPTABLE (no test article description, limited histopathology, no diet analysis, animal husbandry problems). Study superseded by record # 001090 above. A. Apostolou, 5/15/85.
EPA one-liner: Onco NOEL = 100 ppm; no core grade given.

-049, 036398 Partial duplicate of 984561 above.

-044 & 048, 002820 Summary of 984561 above.

-011, 027, 034, & 043, 001124, 001125, 024540, 984565, 984563, & 984557 Supplements (comments on onco effects) to 984561 above.

-049, 036399 Exact duplicate of 001124 (commentary on reexamination of slides of selected tissues of control and 400 ppm females for lymphoreticular tumors concluding no difference between groups).

-049, 036400 Exact duplicate of 024540 (discussion of findings of a series of pathologists).

-065 No record number. "Amitraz: Consideration of the Scientific Issues Relating to the Oncogenic Potential of Amitraz." (Prepared by P. Paul et al, July 8, 1986) Discussion of the oncogenic effect in female B6C3F1 mice in terms of exceeding the MTD at 400 ppm and the influence of hormonal effects. Document should be considered during risk characterization. Gee, 9/22/88.

REPRODUCTION RAT

** -048, 036385 "BTS 27 419: Multigeneration Feeding Test in Rats." (Boots, 9/73) Amitraz (Batch 2099 DH - see # 051314 in 054 for purity of

99.8%), fed in the diet to Wistar rats for 3-generations, 2-litters/generation at 0, 15, 50, or 200 ppm; 10-12 males/group, 20-24 females/group; reduced litter size and substantial neonatal mortality at 200 ppm, slight to moderate neonatal mortality at 50 ppm; originally unacceptable (test article not characterized, no analysis of dosing material, not clear if test article considered to be 100% pure when mixing with food, litters not culled on day four), but upgraded to ACCEPTABLE (with major deviations) by rebuttal and supplemental information. NOEL REDUCED FROM 15 ppm to 10.5 ppm, based on 30% AI content loss in 1 week in the diet, see rebuttal below. F. Martz, 1/13/86, and 7/24/87.

-054, Rebuttal to -048, 036385 (three generation rat reproduction study, Boots, 9/73); supplemental information in 051315, 051316, & 051318 and letter dated 12/5/86. Amitraz (99.8%, see # 051318) to Wistar rats for 3-generations, 2-litters/generation at 0, 15, 50 or 200 ppm in the feed; reduced litter size and substantial neonatal mortality at 200 ppm, slight to moderate neonatal mortality at 50 ppm, NOEL = 15 ppm; originally unacceptable (FM, 1/13/86) mainly because lacked feed analysis. Retrospective stability data (# 051315) showed 30% loss of activity/week with remaining 70% consisting of amitraz and BTS 27 271, a plant and major animal amitraz metabolite; retrospective feed analysis (# 051316) shows that blends can be prepared correctly, albeit on low side of nominal; this information along with reconsideration - study repeat would provide no new useful information to change conclusions or NOEL determination - upgrades study to ACCEPTABLE. NOEL changed from 15 ppm to 10.5 ppm due to feed instability. F. Martz, 7/24/87.

EPA one-liner: LEL = 50 ppm; no grade given.

-012, 015632 Exact duplicate of 036385 above.

SPECIAL REPRODUCTION STUDIES

-008, 984644 "BTS 27 419: Effects on the Estrus Cycle of the Rat." (Boots, 2/72) Amitraz fed in the diet for 18 weeks at 0 or 200 ppm to female rats to examine effects on estrus cycle; 14 in control group, 20 in test group; prolongation of the estrus cycle. A. Apostolou, 5/20/85.

-042, 001108 Duplicate of 984644 above.

-042, 001107 "Amitraz: Investigation of Effects on the Thymus Gland and Oestrous Cycle in Mice." (Boots, test date not evident, 78037) Amitraz technical (97.1%, batch DJ/2703) fed in the diet for 18-33 weeks at 0 or 400 ppm to SPF CFLP female mice to examine effects on estrus cycle; 64 in control group, 52 in exposure group; no adverse effects noted. Follow-up for chronic feeding study--strictly speaking, not a SB950 test type. A. Apostolou, 5/23/85.

-042, 001105 Supplement to 001107 above.

-048, 036394 Summary of 001107 above.

-042, 001109 "Technical Amitraz: The Effect of Dietary Administration on the Oestrous Cycle and Hormones in the Mouse." (FBC, 2/84) Amitraz technical (97.9 - 99.9%) fed in the diet for 28 weeks to B6C3F1 female mice at 0, 25, 100 or 400 ppm; objective was to study effects of test article on hormone levels and estrus cycle; NOEL = 25 ppm; prolongation of pro-estrus

9/23/87

8

phase, trend towards shortening of diestrus phase, prolactin and progesterone serum levels depressed. Not a SB950 test type. A. Apostolou, 2/23/85.

-048, 036395 Summary of 001109 above.

TERATOGENICITY RAT

-048, 036383 "BTS 27 419: Teratogenicity in the Rat." (Boots, 8/73) Amitraz (no purity stated) given by gavage on days 8-20 of gestation to Wistar rats at 0, 1, 3 or 12 mg/kg/day; 11-13 pregnant rats/group; NOEL = 3 mg/kg; intrauterine growth retardation (reduced fetal weights and delayed ossification) at 12 mg/Kg; UNACCEPTABLE (no analysis of dosing solution, no test article characterization, too few animals per group, soft tissue examination appears inadequate), NOT UPGRADABLE. F. Martz, 1/10/86.
EPA one-liner: NOEL = 12 mg/Kg; no grade (Not clear if for 36383 or 984569).

-012, 984571 Exact duplicate of 036383 above.

-051, 044439 Exact duplicate of 036383 above.

-012, 984569 "BTS 27 419: Effect on Pregnancy, Parturition and Care of the Young in Rats." (Boots, 9/73) Amitraz (purity not stated) given by gavage on days 1-20 of gestation to Wistar rats at 0 (0.4% Cellosize), 1, 3 or 12 mg/kg/day; 13-14 pregnant rats/group; no developmental effects reported; NOEL = 12 mg/kg; UNACCEPTABLE (insufficient number of pregnant animals, dosage level not justified, no clinical obs, no neonatal body weight on day 1). Also reviewed by F. Martz as part of 036383 as a preliminary study. A. Apostolou, 5/20/85.

-048, 036384 Exact duplicate of 984569 above.

** 064 065359, 065360 "Technical Amitraz: Teratogenicity Study in the Rat." (Hazleton, UK, TOX 86156, also TOX/87/179-140, 12/87) Technical amitraz, 99.7%, given by oral gavage to 24/group Sprague Dawley Crl:CD(SD)BR rats, 0 (1% methyl cellulose), 7.5, 15.0 or 30.0 mg/kg/day, days 6 - 15 of gestation; maternal NOEL = 7.5 mg/kg/day nominal (decreased body weight gain, decreased food consumption); developmental NOEL = 15.0 mg/kg/day (minor external/visceral defects) - no major malformations or other developmental toxicity due to treatment; no adverse developmental effect; ACCEPTABLE. Record 065360 is analysis of the diet; record # 065358 is the pilot study. Gee, 9/20/88.

-064 065358 "Technical Amitraz: Range-Finding Study in the Pregnant Rat." (Hazleton, UK, 10/87, Project TOX 86154) Amitraz technical, batch CR 20575/3, 99.7%; given by oral gavage days 6 - 15 of gestation to Sprague Dawley CR1:CD(SD)BR female rats, 5 per group at 0 (1% methyl cellulose), 7.5. 15.0 or 30.0 mg/kg/day; slight decrease in body weight gain and food intake at 30 mg/kg/day; no clinical signs or macroscopic findings related to treatment; no evidence for developmental toxicity; supplemental data for Record # 065359. Gee, 9/20/88.

9/23/88

Summary: The possible adverse effect noted in the 1973 study at 12 mg/kg/day was not confirmed in the 1987 study. The overall conclusion is that amitraz did not cause developmental toxicity in the rat. Gee, 9/22/88.

TERATOGENICITY RABBIT

-048, 036388 & 036382 "BTS 27 419: Teratogenicity in the Rabbit." (Boots, 8/73) Amitraz (no purity given) by gavage on days 6-18 of gestation to New Zealand White rabbits at 0 (0.4% Cellosize), 1, 5 or 25 mg/kg/day; 8-10 pregnant animals per group; data quality inadequate to assess NOEL; abortion, fetotoxicity and teratogenicity at high dose level, suspected teratogenicity at the intermediate dose level; UNACCEPTABLE (many major deficiencies including no characterization of test article, no analysis of dosing solution, inadequate numbers of animals, intercurrent respiratory disease), NOT UPGRADABLE. F. Martz, 1/9/86.

EPA one-liner: Teratogenic NOEL > 25 mg/Kg/day; fetotoxicity NOEL = 1 mg/Kg/day; no core grade given.

-012, 984570 Exact duplicate of 036388 & 036382 above.

-048, 002824 Summary of 036388, 036383 and 984569.

** 064 065362, 065363 "Technical Amitraz: Teratogenicity Study in the Rabbit." (Hazleton, UK, TOX/86157, T/278, TOX/87/179-138, 12/87) Amitraz technical, batch CR 20575/3, 99.7%, given by oral gavage to 16 New Zealand White rabbits per group at 0 (1% methyl cellulose in water), 3, 6 or 12 mg/kg/day, days 7 to 19 of gestation; maternal toxicity at all doses noted by clinical signs, reduced body weight gain and food intake at 12 mg/kg/day with 2 aborting and 3 with total litter resorption; maternal NOEL < 3 mg/kg/day, developmental NOEL = 6 mg/kg/day (resorptions, abortions); no major malformations related to treatment; dosing suspension analyses in # 065363; ACCEPTABLE. Gee, 9/21/88.

064 065361, 065363 "Technical Amitraz: Range-finding Study in the Pregnant Rabbit." (Hazleton, UK, TOX 86155, T292, TOX/87/179-136, 10/87) Technical amitraz, 99.7%, given by oral gavage to 5 mated New Zealand White rabbits per group at 0 (1% methyl cellulose in water), 7.5, 15.0 or 30.0 mg/kg/day, days 7 - 19 of gestation; 2/5 aborted at 30 mg/kg/day, 1 died, and 1 had total litter loss at day 28, 4/8 fetuses of the surviving litter at 30 mg/kg had major external malformations with 3 having rudimentary tails and the 4th, acaudia; no other developmental effects were reported; mean fetal weight was reduced at 30 mg/kg/day, maternal body weight gain and food intake were reduced and clinical signs of lethargy and ataxia were noted; supplemental data for # 065362. Gee, 9/21/88.

Summary: The possible adverse effect noted in the 1973 study was not confirmed in the 1987 study in the same strain of rabbits. The overall conclusion is that amitraz did not cause developmental toxicity in rabbits. Gee, 9/22/88.

9/23/88

GENE MUTATION

Microbial Systems

** -042, 001119 "Technical Amitraz: Ames Bacterial Mutagenicity Test." (Inveresk, Scotland, 11/83) Amitraz (98.4%) tested with Salmonella strains TA98, 100, 1535, 1537 and 1538 at 0 (acetone), 33, 100, 333, 1000, 3300 or 10,100 µg/plate; +/- arochlor 1254-induced liver activation; precipitation at 333 µg/plate and above; triplicate plates, three trials; no increase in mutation rate reported; ACCEPTABLE. A. Apostolou, 5/17/85, second opinion by J. R. Gee, 8/5/86.

-042, 001111 "Technical BTS 27 271 Ames Bacterial Mutagenicity Test." (Huntingdon Research Centre, 9/83, FSB 61A/83580) BTS 27271 technical (amitraz metabolite, N-(2,4-dimethyl phenyl)-N-methyl) tested with Salmonella strains TA98, 100, 1535, 1537 and 1538 at 0 (DMSO), 50, 150, 500, 1500 and 5000 µg/plate; +/- rat liver activation; triplicate platings, two trials; no increase in mutation rate reported; UNACCEPTABLE (no protocol). A. Apostolou, 5/22/85, second opinion by J. R. Gee, 8/5/86.

-048, 002819 Summary of 001111 and 001119.

-042, 028979 "Technical BTS 27 919 Ames Bacterial Mutagenicity Test." (Huntingdon Research Centre, 9/83, FSB 61B/83581) Amitraz metabolite, tested on Salmonella strains TA98, 100, 1535, 1537 and 1538 at 0 (DMSO), 50, 150, 500, 1500 and 5000 µg/plate; controls same as in record #001111; no increase in mutation rate reported; UNACCEPTABLE (no protocol), A. Apostolou, 5/22/85, second opinion by J. R. Gee, 8/5/86.

-012, 984574 "BTS 27 419, BTS 27 271, BTS 27 919 and BTS 2B 369: Mutagenicity Testing in Bacterial in vitro Systems." Lab and test date not indicated. Amitraz (purity not indicated) and metabolites tested on Salmonella strains TA1535, 1537 and 1538 and E. coli strains WP2 and WP2 uvrA⁻ at 62.5, 125, 250, 500 and 1000 µg/plate +/- S9; duplicate platings; no increase in mutation rate reported; UNACCEPTABLE (incomplete description of procedures, no repeat trials, E. coli tests without S9), NOT UPGRADABLE. A. Apostolou, 5/17/85.

-042, 001116 "Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems." (Mutation Res. 116:185-216 (1983). Multiple pesticides tested in Ames assay; Amitraz was negative, no data. A. Apostolou, 5/21/85.

Mammalian Systems

** -042, 001115 "Technical BTS 24 868 (2,4-xylidene: Mouse Lymphoma Mutation Assay." (Inveresk, 6/83, Report 2649) BTS 24,868 (2,4-xylidene, 2,4-dimethyl aniline), a metabolite of amitraz, tested on mouse lymphoma cell strain L5178Y at 1, 3.3, 10, 33.3 or 100 ug/ml with mouse liver activation and 0 to 600 ug/ml without activation; 3 hr exposure; two trials; test article increased mutation frequency in presence of S9; ACCEPTABLE. A. Apostolou, 5/21/85, second opinion by J. R. Gee, 8/5/86.

-048, 036391 Summary of 001115 above.

** -042, 001114 "Technical Amitraz: Mouse Lymphoma Mutation Assay." (Inveresk, 9/83, No. 2669) Amitraz (98.4%) tested on mouse lymphoma cell strain L5178Y at 0, 0.2, 0.6, 2, 6, and others to 33 µg/ml +S9 and 0 to 20 µg/ml, -S9 for 3 hrs; with and without mouse liver activation; no consistent increase in mutation frequency reported; ACCEPTABLE. A. Apostolou, 5/20/85, second opinion by J. R. Gee, 8/5/86.

-048,0 36392 Summary of 001118 above.

SUMMARY: The study using the active ingredient, amitraz, does not report an increase in mutation frequency. In contrast, the study on the metabolite, 2,4-xylidene, does report a concentration dependent increase. In evaluating the biological significance of this effect, the in vivo metabolism of amitraz must be considered. The studies reviewed under SB950 do not normally include metabolism studies. For risk assessment, metabolites would be included, if available. Note that other possible adverse effects are listed in this Toxicology One-Liners and Discussion section. Martz, 7/27/87.

CHROMOSOMAL ABERRATION

-042, 001114 "Micronucleus Study in Mice using BTS 24 868." (FBC, 9/83) BTS 24,868 (2, 4-dimethyl aniline--98.8% purity), an amitraz metabolite, given by gavage to CD-1 mice at 56.3, 112.5 or 225 mg/Kg with negative and positive controls for micronucleus assay; two doses separated by 24 hrs; 10 males in positive control group, 5 males in all other groups; only one sacrifice time after second dose at 6 hrs.; no adverse affect reported; UNACCEPTABLE (only males tested, too few animals per group, no justification of dose levels, only one sampling time after dosing, no criteria for scoring), NOT UPGRADABLE. A. Apostolou, 5/21/85, second opinion by J. R. Gee, 8/5/86.

-048, 002818 Summary of 001114 above.

-054, 051319 "Dominant Lethal Assay of Amitraz in the Female Mouse." (Huntingdon Research Centre, 3/25/77) Amitraz, Batch No. DJ 2703, no purity stated, given by oral gavage on five consecutive days to 48 female CFLP mice at 0 (0.4% Cellosize), 12 or 50 mg/kg/day; mated 1:1 with untreated males at 12/group, days 3-8, 9-13, 14-18 or 19-23 post dosing; mean values for embryonic deaths within range of historical controls although a slight trend for an increase in early deaths in 19-23 day group at 50 mg/kg/day; UNACCEPTABLE (no purity of test material, no analysis of dosing solution, inadequate number of females per group, dosing of females instead of males, no justification of doses with no toxicity reported). No dominant lethal effect at the doses tested. J. R. Gee, 7/27/87.

-054, 051320 "Dominant Lethal Assay of Amitraz in the Male Mouse." (Huntingdon Research Centre, 3/29/77) Amitraz, no purity stated, Batch No. DJ 2703; twenty males per group were treated with 0 (0.4% Cellosize), 12 or 50 mg/kg/day for five consecutive days; mated 1:1 with untreated females starting 2 days after last treatment; matings were for 1 week for 6 weekly periods; females examined daily and the day of mating recorded; historical laboratory control mean and range included; no consistent evidence for a dominant lethal effect reported; slight effect on male body weight during dosing in treated groups (4 to 7% lower weight than control); UNACCEPTABLE (no purity of test material, no justification of dose selection with minimal signs of toxicity,

no analysis of dosing solutions); POSSIBLY UPGRADABLE with submission of the missing data. J. R. Gee, 7/27/87.

A replacement study for in vitro human chromosome analysis with technical amitraz is proposed in 287-065, reference 4. Gee, 9/22/88.

DNA DAMAGE

** -042, 001117 "Technical Amitraz: Unscheduled DNA Synthesis in Human Embryonic Cells." (Inveresk, 10/83, Report 2634) Amitraz (100%) tested on human embryonic lung fibroblasts (Flow 2002) at 0, 20, 60, 100, 140, 180, 220 or 260 ug/ml with positive controls in UDS assay; +/- rat liver activation; precipitation and cytotoxicity at 300 ug/ml; duplicate cultures exposed for 3 hrs in the presence of 2.5 mM hydroxyurea and 10 uCi [^{3}H]-thymidine; 50 nuclei/culture were scored for grain counts; negative of UDS; ACCEPTABLE. A. Apostolou, 5/21/85, second opinion by J. R. Gee, 8/5/86.

-048, 036390 Summary of 001117 above.

** -042, 001112 "Technical BTS 24 868 (2,4-xylidene): Induction of Morphological Transformation in C3H/10T1/2 Cells." (Inveresk, 2/84, No. 2835) BTS 24,868 (2,4-xylidene; 2,4-dimethyl aniline--99.2% purity, CR 20391/1), an amitraz metabolite, tested on mouse C3H/10T1/2 cells at 0, 5, 10 or 20 ug/ml with positive and negative controls for transforming capacity + mouse liver activation and 0, 100, 200 or 400 ug/ml without activation; 24 hr exposure; types II and III foci scored after 8 weeks; initially reviewed as incomplete (table 4a of data missing); therefore unacceptable but upgradabale. A. Apostolou, 5/22/85, second opinion by J. Gee, 8/5/86.
-065, 059540 Missing table for 001112, upgrading the study to ACCEPTABLE status.

-048, 036393 Summary of 001112 above.

** -042, 001113 "Technical Amitraz: Induction of Morphological Transformation in C3H10T1/2 Cells." (Inveresk, 9/83, Report 2625) Amitraz (100%) tested on mouse C3H/10T1/2 cells at 12.5, 25 and 37.5 ug/ml + rat liver S9 with positive and negative controls for transforming activity and 0, 5, 10 or 15 ug/ml -S9; 24-hr exposure, 8-week growth period; 11-12 flasks/conc.; negative for transforming activity; ACCEPTABLE. A. Apostolou, 6/10/85, second opinion by J.Gee, 8/5/86.

NEUROTOXICITY

Not required at this time.

9/23/88