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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

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OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: California Department of Food and Agriculture - EPA Toxicology Review for NAPTALAM (Alanap) (Tox. Chem. # 592)

FROM: Alan C. Levy, Ph.D. Alan C. Levy 1-18-89
Toxicologist, Review Section I, Toxicology Branch II
HED (TS-769C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 1/18/89
Acting Branch Chief, Toxicology Branch II
Health Effects Division (TS-769C)

TO: William Burnam, Acting Division Director
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:

STUDY TYPE: Chronic Toxicity (Oncogenicity) Rat - "104-Week Chronic Toxicity in Rats," Hazleton Laboratories, 5/20/81.

Deficiency: "Dose selection not justified, missing individual data for mortality, necropsy, organ weights, purity not stated, no eye exam. Additional data received by CDFA containing individual body weight, food consumption and clinical observation data." "Still considered unacceptable due to lack of other requested information mentioned above. Not upgradable as a combined (chronic & oncogenicity) study (no eye exam)."

EPA Response: The EPA document, "Guidance for the Reregistration of Pesticide Products Containing Naptalam," March, 1985, indicated that the Agency did not have data to satisfy this requirement. Footnote for Guideline §83-2, Oncogenicity Study, stated, "The listed study fulfills part of the data requirement, because it is a valid study using mice. A second study using another species (preferably the rat) is required."

No record of an additional rat toxicity/oncogenicity study in the Agency was located at this time. Therefore, EPA has no comment to make regarding the CDFA deficiencies.

CONCLUSION: Not applicable.

CORE-GRADE: Not applicable.

STUDY TYPE: Chronic Toxicity Dog -

Deficiency: "No study on file." "A new dog study is stated to be in progress at Tegeris Labs, Laurel, Maryland, and due July, 1988."

EPA Response: The EPA document, "Guidance for the Reregistration of Pesticide Products Containing Naptalam," March, 1985, indicated that the Agency did not have data to satisfy this requirement.

No record of a dog toxicity study in the Agency was located at this time.

CONCLUSION: Not applicable.

CORE-GRADE: Not applicable.

STUDY TYPE: Oncogenicity Mouse - "Lifetime Carcinogenicity Study in Mice," IRDC, Mattawan, MI, 8/24/82.

Deficiency: Absence of clinical observation data and an explanation for the variations in test material content in the diet at low dose. CDFA indicated that these have been requested.

EPA Response: The lack of clinical observations (Guidelines state they are to be recorded) is not felt to be a strong enough deficiency to make the study "unacceptable". This determination is based on the primary purpose of the study which is to determine oncogenicity. There were findings of liver hypertrophy of centrilobular parenchymal cells at the mid dose of 2500 ppm. The Agency does, however, feel that the request for these data and their review would add to the completeness of the study.

The Agency agrees with CDFA that the analyses of the low-dose diet indicate larger than usual variations. An explanation is considered to be of relative "academic" interest only, as, if the values are correct, the lowest dose tested is greater than the 50 ppm noted and that this was considered the NOEL.

CONCLUSION: Do not concur with California that this study is a data gap.

CORE-GRADE: Remains unchanged, minimum.

STUDY TYPE: Reproduction Rat - "Multigeneration Evaluation of Alanap Technical in the Sprague Dawley Rat," Food & Drug Research Laboratories, 1/11/80.

Deficiency: No dose justification and no evidence of an MTD.

EPA Response: The above deficiencies were noted in reviews dated 4/4/88 and 6/15/88. In a review dated 12/15/86, the following was noted by CDFA, "Dose selection is justified with a 90-day subchronic feeding study (study not identified)."

CDFA reviews

The EPA document, "Guidance for the Reregistration of Pesticide Products Containing Naptalam," March, 1985, indicated that the Agency did not have data to satisfy this requirement.

Footnote for Guideline §83-4, Reproduction, stated, "Data must be submitted no later than twenty-two months after the publication of this Standard."

No record of an additional rat reproduction study in the Agency could be located at this time. Therefore, EPA has no further comments to make regarding the CDFA deficiencies.

CONCLUSION: Not applicable

CORE-GRADE: Not applicable

STUDY TYPE: Teratogenicity, Rat - "Teratologic Evaluation of Alanap Technical in Sprague-Dawley Rats," Food & Drug Res. Labs., 12/22/78.

Deficiency: On the "Summary of Toxicology Data" sheet, it is stated that "No adverse effect indicated." Within the CDFA document, UNACCEPTABLE appears and refers to, "no definition of the "X" notation supposedly described on reverse of page but reverse was blank, resorptions not given as "early" and "late", no analysis of dosing solution." In addition, the following appears, "Individual data and replacement pages with copy of reverse side and explanation of "X" notations. The 1978 guidelines did not specify that resorptions should be determined as early and late so the protocol did not require this distinction."

EPA Response: The maternal NOEL was 15 mg/kg (doses of 0, 15, 115 or 500 mg/kg); mortality occurred and body weight was affected. There was no apparent developmental toxicity without maternal toxicity. A teratogenicity study may be acceptable without developmental toxicity.

The EPA document, "Guidance for the Reregistration of Pesticide Products Containing Naptalam," March, 1985, indicated that the rat teratogenicity study was valid.

CONCLUSION: Do not concur with California that this study is a data gap.

CORE-GRADE: Based upon the "Registration Standard," this has been considered "valid" (currently termed core minimum).

STUDY TYPE: Teratogenicity, Rabbit - "Teratology Study in Rabbits", IRDC, 5/31/85.

Deficiency: No adverse effect indicated, no evidence of developmental toxicity at any dose. Number of pregnant females is less than guidelines for mid- and high-dose groups.

EPA Response: Maternal toxicity of reduced body weight, mortality and clinical observations at the HDT of 650 mg/kg/day. Developmental LOEL of 650 mg/kg/day (increased numbers of fetuses and litters with forked scapula, hyoid arches and misaligned sternebrae).

EPA agrees that the number of pregnant females is less than guidelines for the mid- and high-dose groups. However, the EPA reviewers felt that the study was acceptable because of a maternal effect, a developmental effect and sufficient offspring in the mid- and high-dose groups to make an adequate scientific evaluation of the potential developmental effect of the chemical.

CONCLUSION: Do not concur with California that this study is a data gap.

CORE-GRADE: Remains unchanged, minimum.

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

NAPTALAM, SODIUM SALT (ALANAP)

SB 950-271, Tolerance # 297

December 16, 1986
Revised April 5, 1988
Revised June 24, 1988

I. DATA GAP STATUS

Combined (chronic + onco) rat:	Data gap, inadequate study, no adverse effect indicated
Chronic dog:	Data gap, no study on file, study in progress
Onco mouse:	Data gap, inadequate study, possible adverse effect indicated
Repro rat:	Data gap, inadequate study, no adverse effect indicated
Terato rat:	Data gap, inadequate study, no adverse effect indicated
Terato rabbit:	Data gap, inadequate study, no adverse effect indicated
Gene mutation:	No data gap, no adverse effect
Chromosome:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotox:	Not required at this time

Note, Toxicology one-liners are attached

** indicates acceptable study
Bold face indicates possible adverse effect
File name T880620
Toxicology Summary update by M. Silva, 6/88.

II. TOXICOLOGY SUMMARY

COMBINED (CHRONIC + ONCO) RAT

008 37181 "104-Week Chronic Toxicity in Rats," (Hazleton Laboratories, 5/20/81). Naptalam, "assumed" 100%, but purity and lot number were not provided; 50/sex/group fed 0, 120, 600 or 3000 ppm over 104 weeks; NOEL = 600 ppm (female body weight). **No adverse effect.** The report was originally reviewed as **unacceptable** (dose selection not justified, missing individual data for mortality, necropsy, organ weights, purity not stated, no eye exam) by K. Pfeifer, 7/12/85 and J. Gee, 7/16/86, 12/15/86. CDFA received DPN/Volume/Record#: 297/017/57581 which contained individual body weight, food consumption and clinical observation data. The study remains **unacceptable,** however because it still lacks other requested information mentioned above. **Not upgradeable as a combined (chronic & oncogenicity) study** (no eye exam). D. Shimer, 2/24/88. M. Silva, 6/15/88.

001 17029 Summary of 37181

016 Appendix B. "Subacute Dietary Administration - Rats." (Hazleton, 5/10/68.) Justification of the doses used in 37181. Thriteen-week feeding study at 0, 500, 1000 or 5000 ppm. Subchronic study suggests the NOEL in males $\geq$ 5000 ppm with marginal effects on body weight in females at that dose. McGee and Gee, 12-16-86.

016 Appendix C. Replacement pages 124-155 for 37181.

017 57581 This volume contains individual body weight, food consumption and clinical observation data for 008 37181. M. Silva, 6/22/88.

CHRONIC DOG

Subchronic, Dog

017 57586 "Alanap 30-Day Dose Range Finding Oral Toxicity Study in the Dog," (Tegeris Laboratories Inc., project no. 86065, 2-27-87). Alanap technical (lot DJS-1-65-A; purity = 89.4%) was given to Beagle dogs in the feed at 0, 1000, 4000 and 8000 ppm for 30 days, 2/sex/group. All animals were necropsied and tissues required by the guidelines were saved but were not examined for histopathology. Fortified feed was found to contain the proper amount of test article which was stable for 7 days. **No adverse effects.** NOEL = 1000 **ppm** (reduced body weight gain and feed consumption at 4000 and 8000 ppm in both sexes; one mid dose male had increased gamma glutamyl transpeptidase correlating with thickened and lobulated gallbladder). **Supplemental study** (range-finding for dog chronic). D. Shimer, 3/28/88. M. Silva, 6/15/88.

016 Appendix D. A new dog study is stated to be in progress at Tegeris Labs, Laurel, Maryland, and due July, 1988.

M. Silva 6/25/88
J. Parker 6-27-88

ONCOGENICITY RAT

No study on file. See comments under Combined Rat.

ONCOGENICITY MOUSE

006, 007 37179 37180 "Lifetime Carcinogenicity Study in Mice," (IRDC, Mattawan, MI, 8/24/82). Naptalam (purity = 92%) was used on Charles River CD-1 mice (75/sex/group) at 0, 50, 2500 or 5000 ppm for 84 weeks. NOEL = 50 ppm (liver hypertrophy in both sexes at 2500 and 5000 ppm). No oncogenic effect was clearly identified. Problems with low dose diet analysis showing 400-500 ppm in week 15 and >150% in several samplings at the end of the study suggesting that 50 ppm is a conservative NOEL. The incidence of liver carcinomas in males at 5000 ppm (10%) is outside of the ranges of 3 pooled historical controls but is not significant compared with the concurrent control by Fisher's Exact Test. The study was originally reviewed as unacceptable but possibly upgradeable with submission of food consumption and clinical observation data (Pfeifer, 7/11/85 and Gee, 7/15/85 and 12/15/86). CDFA received DPN/Volume/Record#: 297/018/57587 which contained only the individual food consumption data. Therefore the study remains **unacceptable but upgradeable** (clinical observation data and an explanation for the variations in test material content in the diet at low dose are requested). D. Shimer, 4/4/88. M. Silva, 6/15/88.

001 17028 Summary of 37179, 37180.

016 Appendix E. Purity of Alanap for 37179 was 92%.

016 Appendix F. Protocol with corrections.

018 57587 This volume contains individual food consumption data for study 006 37179 and 007 37180.

ONCOGENICITY, DOG

001 17027 "Nine-Year Feeding Study of ANA in Dogs for Tumor Induction." (Uniroyal Chemical, 5/20/77) Interim summary of oncogenicity after 9 years of treatment. Doses stated to be 400 mls/day. Insufficient information for assessment. Pfeifer, 7-11-85.

REPRODUCTION, RAT

009 37182 "Multigeneration Evaluation of Alanap Technical in the Sprague Dawley Rat," (Food & Drug Research Laboratories, 1/11/80). Naptalam technical (sodium salt; purity = 91%--two lots used) was fed to Sprague Dawley rats (20/sex/group--F0; 25/sex/group F1) at 0, 120, 600 or 3000 ppm (diets not corrected for purity) for 3 generations (1 litter/generation). Parental NOEL > 3000 ppm (no effects observed at any dose). Reproductive NOEL = 600 ppm (decreased pup weight gain). Originally reviewed as unacceptable (no gross or histopathology on F0 adults; and since no day 7 or day 14 pup weights were taken, the significance of the lower weight gain at 3000 ppm on day 21 cannot be adequately evaluated; no individual data, no justification of dose with no evidence of an MTD) by Pfeifer, 7/11/85 and Gee 7/17/86. CDFA received DPN/Volume/Record#: 297/019/57588 which contained individual daily clinical observations, individual gestation and lactation body weight data, individual mating records, parturition data, litter data (pup body weight was by litter, not individual), and litter weights on days 1, 4, and 21. Upon re-review it

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was also noted that although gross and histopathology were missing on the F2 pups, enough data were provided by having 5/sex/dose examined for F1 and F3. In addition, tissues from the control and 3000 ppm F1 and F2 adults and all dose levels of the F3 generation were examined for gross and histopathology. The study remains **unacceptable** and **not upgradeable**, however, primarily because there was no dose justification and no evidence of an MTD. D. Shimer, 4/4/88. M. Silva, 6/15/88.

001 17024 Summary of 37182.

016 Appendix G. Response of FDRL to CDFA review. Uniroyal will be submitting additional individual data. Protocol did not require necropsy of the F0 adults so none was performed. Animals were fed diets for 10 weeks prior to mating. Analyses of diets are included with 7-day stability data. Dose selection is justified with a 90-day subchronic feeding study (study not identified). Pup weights on day 14 were not required by the protocol. Nonetheless, these data would greatly assist in determining whether an adequate high dose was used since the major finding was decreased weight gain of pups between days 4 and weaning. No interim weights were recorded. J. Gee, 12/15/86.

019 57588 This volume contained individual daily clinical observations, individual gestation and lactation body weight data, individual mating records, parturition data, litter data (pup body weight was by litter, not individual), and litter weights on days 1, 4, and 21. These data pertain to study 009 37182. M. Silva, 6/19/88.

TERATOGENICITY, RAT

010 37183 "Teratologic Evaluation of Alanap Technical in Sprague-Dawley Rats." (Food & Drug Res. Labs., 12/22/78) Naptalam, sodium salt, Technical, 91.3%; Rats were given 0, 15, 115 or 500 mg/kg, days 6-15 by gavage; 23-36 pregnant dams per group; aspirin as positive control; NOEL (maternal weight, mortality) = 15 mg/kg, no developmental toxicity without maternal toxicty - NOEL = 115 mg/kg; **UNACCEPTABLE** (no definition of the "x" notation supposedly described on reverse of page but reverse was blank, resorptions not given as "early" and "late", no analysis of dosing solution.) Pfeifer, 7-12-85 and Gee, 7-18-86.

001 17023 Summary of 37183.

016 **Appendix H.** Individual data and replacement pages with copy of reverse side and explanation of "x" notations. The 1978 guidelines did not specify that resorptions should be determined as early and late so the protocol did not require this distinction.

016 Appendix I. Purity of test article was 91.3%.

TERATOGENICITY, RABBIT

013 43302 "Teratology Study in Rabbits." (IRDC, 5/31/85.) Naptalam, sodium salt, 92%, Lot # 3199300; 16 does/group given 0, 50, 200 or 650 mg/kg/day, by oral gavage days 7 - 19; maternal NOEL = 50 mg/kg (body weight); no evidence of developmental toxicity at any dose - developmental

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NOEL ≥ 650 mg/kg. **UNACCEPTABLE**, possibly upgradeable but number of pregnant females is less than guidelines for mid- and high-dose groups. Gee, 7-18-86.

016 Appendix J. Purity of test article was 92%. No analysis of dosing solution was done.

GENE MUTATION

** 017 57584 "CHO/HGPRT in vitro Mammalian Cell Mutation Assay on Sodium Alanap," (American Biogenics Corporation, 1-5-87). Sodium alanap (lot DJS-050586; purity = 91.8%) was tested in the CHO/HGPRT assay with and without S9 activation (duplicate flasks). With activation, cells were exposed for 4 hours at doses of 14.9, 29.8, 49.8, 99.6, 149, 298, 498 and 996 ug/ml (concentrations of ≥ 996 ug/ml were toxic, with 84% survival at 498 ug/ml). Without activation, cells were exposed for 16 hours at concentrations of 15, 30, 50, 100, 150, 300, 500, 1000 and 1500 ug/ml (at 1500 ug/ml there was survival in only one flask due to toxicity). Positive controls (DMBA-activation and EMS-no activation) functioned as expected. No increase in mutants was observed. **Acceptable.** Shimer, 3-7-88. M. Silva, 6-16-88.

001 33583 "Evaluation of Herbicides for Possible Mutagenic Properties - Point Mutations using Salmonella typhimurium, and T4 Bacteriophage in E. coli." (Columbus Labs., Batelle Memorial Institute, 1972.) Salmonella and E. coli T4 bacteriophage AP72 and N17. No concentrations given. Summary only. **UNACCEPTABLE.** Negative for mutagenicity. Pfeifer, 7-11-85.

001 17025 Summary of 37184.

011 37184 "Mutagenicity Evaluation of Alanap Technical in the Ames Salmonella/microsome plate test." (Litton Bionetics, 10/78) Salmonella; Naptalam technical, no purity stated; strains TA1535, TA1537, TA1538, TA98 and TA100 with and without rat liver S9 activation; tested at 0, 1.0, 10, 100, 500, 1000 or 2000 ug/plate; 1 plate per concentration, repeat trial for TA1537 and TA100. **UNACCEPTABLE** (no purity of test article, apparently a single plate per concetration, repeat trial with 2 strains only, no good justification for 2000 as maximum amount - colony counts do not reflect cytotoxicity.) Possibly upgradeable. Gee, 7-15-86.

MUTAGENICITY, CHROMOSOME

** 017 **57582** "Micronucleus Assay With Sodium Alanap," (American Biogenics Corporation. 12-9-86). Sodium alanap (batch no. DJS-050586· purity = 91.8%) was given by gavage to CD-1 mice, 5/sex/time point/group. Dose levels were 500, 750 or 1500 mg/kg and animals were sampled at 1,2 and 3 days. Micronuclei were counted only at the high dose. Two males died at 1500 mg/kg, 1 male died at 750 mg/kg. No increase in number of micronucleated erythrocytes. Positive control (cyclophosphamide) functioned as expected. **Acceptable.** Shimer, 2-29-88. M. Silva, 6-15-88.

** 017 **57583** "In Vitro Chromosomal Aberration Assay on Sodium Alanap," (American Biogenics Corporation, December 8, 1986). Sodium Alanap (lot DJS-050586; purity = 91.8%) was used on Chinese hamster ovary cells at 298, 497, 995, 1490 and 2990 ug/ml (no activation--8 or 17 hour exposure) or 257, 771, 1540, 2570 and 5140 ug/ml (with activation--2 hour exposure), then cells were

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grown for 8 or 17 hours. Positive controls were cyclophosphamide and mitomycin C and they functioned as expected. All concentrations were tested in duplicate flasks. 50 metaphase cells/concentration were scored. Naptalam was toxic in the nonactivated assay at ≥ 2990 ug/ml. With activation it was toxic at 5140 ug/ml. **Adverse effect** (An increase in chromosomal damage was observed with activation at ≥ 1540 ug/ml and without activation at 1490 ug/ml after a 17 hour growth period). **Acceptable.** Shimer, 2-29-88. M. Silva, 6-16-88.

Conclusion: The results of the micronucleus test (record #: 57582) showed no adverse effect while there was an adverse effect with the chromosomal aberration test (record #: 57583). Based upon the number of mice killed in the micronucleus test range-finding, the maximum dose was administered. However, since no increase in %PCE was observed in the definitive test, there was also no evidence that naptalam actually reached the bone marrow. On the other hand, there is no assurance that the bone marrow is a target tissue. It is the conclusion of CDFA, therefore, that the findings from the chromosomal aberration test (record #: 57583) should be considered in the evaluation of the possible toxic effects of naptalam.

MUTAGENICITY, DNA/OTHER

** 017 57585 "Unscheduled DNA Synthesis in Rat Primary Hepatocytes Test Article Sodium Alanap," (Microbiological Associates, T5270.380, 3-31-87). Sodium alanap (lot DJS-050586; purity = 91.8%) was tested for UDS by autoradiographic methods with rat liver cells from an adult male Sprague-Dawley rat at concentrations of 3, 10, 30, 100, 300, 1000, 3000, and 10,000 ug/ml (3 plates/concentration for UDS; 2/concentration for parallel cytotoxicity). Cells were treated for 18-20 hours and 25/plate were counted. A precipitate was seen at the top 5 concentrations, visual observations indicated cytotoxicity at the 2 highest concentrations. Positive control (DMBA) functioned as expected. **No adverse effects** (no increase in unscheduled DNA synthesis was observed at any dose). **Acceptable.** Shimer, 3-28-88. M. Silva, 6-16-88.

NEUROTOXICITY

Not required at this time.

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Chemical:	**Benzoic acid, 2-((1-naphthalenylamino)ca**
PC Code:	**030702**
HED File Code	**13000 Tox Reviews**
Memo Date:	**01/18/89**
File ID:	**00000000**
Accession Number:	**412-03-0114**

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