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

008592

SEP 13 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of "A 21-Day Dermal Toxicity Study of Mesurol Technical in Albino Rabbits."

Tox.Chem No.: 578B
MRID No.: 417717-01
HED Project No.: 1-1127
Submission No.: S394833

To: Nancy Tompkins
SRR Branch
SRR Divison (H7508C)

From: John C. Redden, Toxicologist 8/30/91
Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

Thru: Henry Spencer, Ph.D. 8/30/91
Acting, Section Head Section 3
Toxicology Branch 1
Health Effects Division (H7509C) KB 8/31/91

ACTION:

The registrant has submitted for review MRID No. 417717-01 "A 21-Day Dermal Toxicity Study of Mesurol Technical in Albino Rabbits."

CONCLUSIONS:

Mesurol technical was applied to the dorsal skin area of rabbits of both sexes at 500 mg/kg, 6 hours a day for 21 days. Decreased food consumption and reduced growth in female rabbits were the primary treatment-related changes. There was a significant decrease in plasma cholinesterase activity of females on day 14. This change was not observed at termination. The study is classified as Core supplementary as it does not meet the requirements of Guideline § 82-2. Only one dose was used and this dose did not meet the limit dose test of 1000 mg/kg. The method for cholinesterase analysis was not submitted with the study, nor

were the analyses methodology and results for compound purity. The individual animal data for dates of when backs were shaved, and clinical signs were not submitted. NOEL and LOEL cannot be determined for the study without the additional data noted as unsubmitted.

EPA No.: 68D80056
DYNAMAC No.: 384-A
TASK No.: 3-84A
August 7, 1991

DATA EVALUATION RECORD

MESUROL

21-Day Subchronic Dermal Toxicity Study in Rabbits

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: William L McFulan for

Date: Aug 7, 1991

CASWELL FILE

EPA No.: 68D80056
DYNAMAC No.: 384-A
TASK No.: 3-84A
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DATA EVALUATION RECORD

MESUROL

21-Day Subchronic Dermal Toxicity Study
in Rabbits

REVIEWED BY:

Aisar Atrakchi, Ph.D.
Principal Reviewer
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Signature: Aisar Atrakchi
Date: August 7 91

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Date: August 7, 1991

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Signature: Henry Spencer
Date: Aug. 29, 1991

Karl Baetcke, Ph.D.
EPA Branch Chief
Toxicology Branch I
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Signature: _____
Date: _____

DATA EVALUATION RECORD

GUIDELINE §82-2

STUDY TYPE: Three-week dermal toxicity study in rabbits.

MRID NUMBER: 417717-01.

TEST MATERIAL: Mesurol technical; 3,5-dimethyl-4-(methylthio)phenol methylcarbamate.

SYNONYMS: Methiocarb, mercaptodimethur, metmercapturon, Bay 37344, H321.

STUDY NUMBER: 51925.

SPONSOR: Mobay Corporation, Health, Environmental and Safety Corporate Toxicology, Stanley Research Center, Stilwell, KS.

TESTING FACILITY: Bio-Research Laboratories Ltd. Senneville, Quebec, Canada.

TITLE OF REPORT: A 21-Day Dermal Toxicity Study of Mesurol Technical in Albino Rabbits.

AUTHOR: B. G. Proctor.

REPORT ISSUED: August 31, 1989.

CONCLUSIONS:

Mesurol technical (97.5%) was applied to the dorsal skin of male and female rabbits at 500 mg/kg, 6 hours/day for 21 days. The control group received 0.9% saline. The main compound-related changes were decreased food intake and reduced growth in female rabbits. No animals died prior to schedule. No changes of any toxicological significance were seen in hematology or clinical chemistry parameters. No compound-related effects on organ weights, gross pathology, or histopathology were noted. There were no effects on erythrocyte or brain cholinesterase, but a significant decrease in plasma cholinesterase activity in females on day 14 of application was noted. This change was not exhibited at study termination. A NOEL value was not derived because only one dose was tested; in addition, only plasma cholinesterase was depressed in one sex, and recovery occurred in those animals.

Classification: CORE Supplemental. This study did not satisfy requirements of Guideline 82-2; only one dose (500 mg/kg) was used, and it did not satisfy the suggested limit test dosage level of 1000 mg/kg. The methods of analysis for erythrocyte, plasma, and brain cholinesterase should be submitted for review.

A. MATERIALS:

1. Test Compound: Mesurol; description: white crystalline powder with mild odor; batch No.: 86I004; purity: 97.5% active ingredient.

2. Test Animals: Species: rabbit; strain: New Zealand White; age: 12 weeks on first day of dosing; weight: males--2.6 to 3.3 kg, females--2.6 to 3.1 kg on first day of dosing; source: Maple Lane Farm, Clifford, Ontario.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated for 15 days to the laboratory environment before testing. Animals were assigned to the following test and control groups based on body weight, using a computer-generated, pseudo-randomization procedure:

Test Group	Dose Level (mg/kg)	Main Study (21 Days) Males	Females
1 Control (0.9% saline)	0	5	5
2 Mesurol Technical	500	5	5

The basis for choosing this dose was not provided by the author. The animals were given a general physical examination immediately after arrival at the test facility. Animals were housed individually in stainless steel cages in a room with the temperature maintained at 17 ± 3°C, relative humidity between 30 to 70%, and a 12-hour light/dark cycle.

2. Dose Preparation: The selected dose of mesurol was prepared daily by dissolving the compound in physiological (0.9%) saline (about 1.5 mL/animal). Mesurol was stored in the dark at room temperature; it was reported to be stable under normal use conditions.

   Results: Analysis techniques to test homogeneity, stability, and percent concentration of mesurol in the solutions were not provided.

3. Preparation of Animal Skin: The fur of each rabbit was shaved from the dorsal area measuring not less than 4 inches in width and 8 inches in length (minimum 10% of body surface). The animals were prepared 24 hours before the start of the study and were shaved as needed thereafter. Using a metal spatula, mesurol was evenly applied to a piece of gauze. The moistened gauze was applied to the shaved skin of the rabbit (compound-treated side down) 6 hours/day for 21 consecutive days. The site of application on each rabbit was occluded with an impervious wrap during exposure. The residual compound was wiped with gauze moistened with tapwater after the end of the 6-hour exposure period. To prevent oral ingestion of the compound, each rabbit wore a flexible Elizabethan collar during the 18-hour intervals between exposures. Physiological saline (0.9%, lot No. AP322N7; expiration date November 1989) manufactured by Travenol Canada Ltd. was applied to the control animals of each sex in the same way as mesurol was applied to the treated animals. The volume of saline was about the same as that used to moisten the gauze containing mesurol. The study author indicated that one rabbit was eliminated from the study because of skin injury at the site of application (shaved area). A second rabbit was also eliminated from the study because of undesirable clinical findings. Both rabbits were replaced.

4. Food and Water Consumption: The diet was a standard pelleted Purina Certified Rabbit Chow No. 5322; water was fresh tapwater. Daily allotments of lettuce and carrots were routinely given to rabbits during the acclimation period and throughout the study period. Food and water were provided to all animals ad libitum except during scheduled fasting periods.

5. Statistics: The study author used statistical analyses to determine group differences in body weights, food consumption, clinical pathology values, brain cholinesterase levels, and absolute and relative organ weights. The study author tested for homogeneity of variance with Bartlett's test ($p < 0.001$). If variances were homogeneous, then parametric methods such as ANOVA (analysis of variance-one way) using the F-distribution were used to analyze group differences. If intergroup variances were different, then the nonparametric Kruskal-Wallis test was used. Furthermore, if mean differences occurred after nonparametric analysis, then a summed rank test was used to determine which group means differed from the control means. Statistical significance was reported when $p < 0.05$.

6. Quality Assurance: A Good Laboratory Practice Compliance (GLP) statement was provided, signed, and dated August 8, 1984, and another GLP statement for the final report was signed and dated November 21, 1989. No quality assurance statement, however, was provided.

C. METHODS AND RESULTS:

1. Observations: All animals were checked twice daily before, during, and after the exposure period, and once daily during the acclimation period. The rabbits were observed for signs of morbidity and mortality. Dermal irritation was scored using the method of Draize (1965); the animals were scored daily prior to start of the experiment and before necropsy.

   Results: None of the animals of either sex died. The only dermal effects were occasional erythema, edema, or focal skin lesions detected in the control as well as the treated animals. These effects were seen toward the end of the study and were considered incidental and unrelated to treatment. Several treated as well as control animals had slight to moderate lacrimation of one or both eyes at different times during the study period. In a few treated as well as control rabbits, periocular alopecia was also noted toward the end of the study period. Two male rabbits (2011 and 2021) showed slight to moderate signs of toxicity on dosing day 10. The signs included ataxia, moist rails, moderate salivation, pupillary constriction, grinding of teeth, decreased motor activity, tachypnea, or dyspnea; rabbit No. 2011 also showed moderate lacrimation in both eyes. These toxicity signs were due to ingestion of mesurol, as these two rabbits managed to remove the dressing after application and, by grooming, ingested the compound. The study author concluded that except for the transient toxicity observed after ingestion of mesurol in

the two rabbits, the clinical findings were not attributed to dosing. The reviewers were not able to confirm clinical findings, since the study author did not provide any individual clinical data.

2. Body Weight: Each rabbit was weighed once prior to onset of the study and twice weekly during the course of the study. Final body weight was measured prior to necropsy after an overnight fast.

   Results: Table 1 presents the mean body weight and body weight gain/loss of both male and female rabbits. Body weights in treated males were similar to those of the control groups. Female body weights, however, averaged less than those of the corresponding controls. A significant ($p < 0.05$) decrease was noted during application days 15, 19, and 21. This effect was suggested to be mesurol-induced.

3. Food Consumption and Compound Intake: Food consumption in each animal was measured every 2 days throughout the study period.

   Results: Mean daily food consumption in male and female rabbits is presented in Table 2. Treated males and females consumed less than the corresponding controls throughout the study. However, statistical significance was only found in males during days 13 to 15 and in females during days 19 to 21. The decrease in female food consumption correlated with the observed decline in body weight. Although these changes were statistically significant, the difference was marginal. The author concluded that the decrease in food intake was compound related.

4. Ophthalmological Examinations: Ophthalmological examinations were not conducted.

5. Hematology and Clinical Chemistry: Blood was collected from the auricular artery one week before end of the study and at study termination (days not specified) from each fasted animal for hematology and clinical chemistry analysis. For blood cholinesterase activity measurement, blood was collected from each control and treated animal before the start and immediately following the end of 6-hour exposure on study days 1, 7, 14, and 21. For the treated animals only, additional samples were collected 16 hours after end of exposure on study days 1, 7, 14, and 21. The checked (x) parameters were examined:

TABLE 1. Mean Body Weight and Body Weight Gain (g ± S.D.) in Rabbits Exposed Dermally to Mesurol for 21 Days[a]

Dose Level (mg/kg)	Mean Body Weight (± S.D) at Days: 1	5	8	12	15	19	21	22
				Males				
0	2.92 ± 0.18	2.80 ± 0.10 (-0.12)[b]	2.98 ± 0.05 (+0.06)	2.96 ± 0.06 (+0.04)	3.0 ± 0.10 (+0.08)	3.06 ± 0.11 (+0.14)	3.14 ± 0.15 (+0.22)	3.08 ± 0.11 (+0.16)
500	2.94 ± .30	2.80 ± 0.32 (-0.14)	3.0 ± 0.28 (+0.06)	3.0 ± 0.26 (+0.06)	2.86 ± 0.37 (-0.08)	3.0 ± 0.34 (+0.06)	3.06 ± 0.36 (+0.12)	3.0 ± 0.37 (+0.06)
				Females				
0	2.84 ± 0.15	2.78 ± 0.13 (-0.06)	2.92 ± 0.16 (+0.08)	2.9 ± 0.12 (+0.06)	3.0 ± 0.15 (+0.16)	3.08 ± 0.15 (+0.24)	3.12 ± 0.19 (+0.28)	3.04 ± 0.17 (+0.2)
500	2.72 ± 0.08	2.66 ± 0.11 (-0.06)	2.76 ± 0.11 (+0.04)	2.8 ± 0.14 (+0.06)	2.76 ± 0.11* (+0.04)	2.86 ± 0.11* (+0.12)	2.86 ± 0.15 (+0.12)	2.86 ± 0.11* (+0.12)

[a]Based on five rabbits/sex/dose.

[b]Numbers in parentheses are the weight gain/loss in g.

*Significantly different from the control value at $p < 0.05$ (Dunnett's test).

TABLE 2. Food Consumption (g ± S.D.) at Selected Intervals During the Study in Rabbits Exposed Dermally to Mesurol[a]

Dose Level (mg/kg)	Food Consumption (± S.D.) at Days: 7-9	13-15	17-19	19-21
		Males		
0	359.2 ± 49.7	379.2 ± 46.5	370.5 ± 28.8	428.6 ± 37.1
500	322.6 ± 60.9	284.8 ± 57.5*	356.0 ± 86.7	402.8 ± 73.2
		Females		
0	341.2 ± 34.7	375.4 ± 55.3	348.0 ± 43.5	408.2 ± 21.0
500	283.8 ± 63.4	347.6 ± 36.7	322.6 ± 78.5	366.8 ± 27.6*

[a]Based on five rabbits/sex/dose.

*Significantly different from control value at $p < 0.05$ (Dunnett's test).

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count†
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)†
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)†
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)†
X	Platelet count†	X	Coagulation:thromboplastin time (PT)†
X	Reticulocyte count (RETIC)		
X	Red cell morphology		

Results: No changes of statistical significance occurred in any of the hematological parameters measured in treated male or female rabbits relative to the corresponding values of the control groups.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
X	Cholinesterase	X	Total protein†
	Creatine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
X	Gamma glutamyltransferase (GGT)		

Results: Table 3 presents selected parameters of clinical chemistry in treated and control groups prior to dosing and at study termination. In males, no statistically significant differences were noted. However, some nonsignificant changes in these males were noted by the reviewers: a 31.8% decrease in SGPT enzyme activity, a 24%

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Selected Clinical Chemistry Parameters (± S.D.) for Rabbits Dermally Exposed to Mesurol for 21 Days

Dose Level (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Triglycerides (mg/dL)	Calcium (mg/dL)
			Males		
0	20.6 ± 4.45 (24.8 ± 5.36)[a]	66.0 ± 16.08 (85.0 ± 30.66)	153.4 ± 16.1 (202.4 ± 29.31)	58.8 ± 25.96 (100.0 ± 36.03)	12.7 ± 0.44 (13.1 ± 0.19)
500	20.2 ± 7.56 (23.4 ± 6.02)	45.0 ± 14.23 (57.8 ± 14.97)	171.0 ± 79.03 (238.4 ± 41.33)	44.6 ± 7.23 (67.2 ± 18.31)	12.9 ± 0.34 (13.2 ± 0.43)
			Females		
0	14.0 ± 3.46 (17.6 ± 4.39)	36.6 ± 7.64 (48.4 ± 8.85)	172.4 ± 73.98 (201.8 ± 58.09)	44.4 ± 13.46 (72.6 ± 20.59)	12.8 ± 0.30 (13.1 ± 0.45)
500	21.6 ± 5.03* (22.0 ± 5.70)	60.6 ± 14.15* (79.8 ± 13.83)**	189.4 ± 15.96 (215.4 ± 18.19)	35.6 ± 5.41 (85.0 ± 42.70)	12.4 ± 0.23* (13.0 ± 0.24)

[a]Numbers in parentheses represent pretreatment values.

*Significantly different from control value, $p < 0.05$ (Dunnett's test).

**Significantly different from control value, $p < 0.01$ (Dunnett's test).

decrease in triglycerides, and a slight (11.5%) increase in alkaline phosphatase were found when compared to concurrent controls (both changes nonsignificant). In females, a significant ($p < 0.05$) increase was noted in the activity of SGOT (54%) and SGPT (66%) enzymes measured at the end of the study as well as a slight (3%) but significant ($p < 0.05$) decrease in the serum calcium level. These findings are without any toxicological significance because the pretreatment levels of SGOT and SGPT in treated female rabbits were higher than their corresponding control values (moreover, SGPT activity of the dosed females prior to dosing showed statistical significance relative to the pretreatment control group).

Table 4 presents the mean plasma, erythrocyte, and brain cholinesterase activity levels in both sexes measured at study termination. Blood and brain cholinesterase activities were not affected by mesurol. The only significant change was found in females in the plasma cholinesterase activity measured at day 14, 6 hours after end of exposure. The effect was a significant ($p < 0.05$) decrease relative to the corresponding control value (460.2 ± 32 vs. 535.0 ± 41.46, respectively). This inhibition did not recover to normal level measured 16 hours later; however, recovery to control values was observed in plasma collected on day 21 (561.4 ± 47.13 vs. 575.4 ± 69.6 in treated and control animals, respectively). In both sexes, the pretreatment blood cholinesterase activity was, in general, higher than that measured after exposure. The toxicological significance of this finding is unclear.

6. Urinalysis: Urinalyses were not performed.

7. Sacrifice and Pathology: Histopathological examination was limited to the kidneys, liver, testes/ovaries, application site, and treated and untreated skin. The following checked (x) organs and tissues were collected for preservation and fixation; those marked with (xx) were also weighed:

TABLE 4. Plasma, Erythrocyte, and Brain Cholinesterase Activity in Male and Female Rabbits Dermally Exposed to Mesurol for 21 Days[a]

Dose Level (mg/kg)	Cholinesterase Activity		
	Plasma (U/L)	Erythrocyte (U/L)	Brain[b] (MU/G)
		Males	
0	517.2 ± 61.63 (507.8 ± 99.08)	3530.6 ± 354.92 (4453.2 ± 2667.01)	6701.2 ± 1001.71
500	525.0 ± 134.94 (548.0 ± 188.85)	3749.4 ± 367.77 (4454.8 ± 1384.34)	7571.6 ± 244.04
		Females	
0	575.4 ± 69.60 (760.4 ± 135.56)	3580.2 ± 422.17 (5700.4 ± 1722.76)	7337.8 ± 361.44
500	561.4 ± 47.13 (730.0 ± 102.55)	4024.6 ± 341.48 (6812.6 ± 467.62)	7091.2 ± 711.63

[a]Values for plasma and erythrocyte cholinesterase are those measured on day 21 immediately after the end of the 6-hour exposure; numbers in parentheses are pretreatment values.

[b]For brain cholinesterase, the values are collected from the left half of the brain following immediate necropsy of the tissues.

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve (sciatic nerve)
X	Esophagus	X	Bone marrow	X	Spinal cord (cervical level)
X	Stomach	X	Lymph nodes	XX	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		
X	Ileum				
X	Cecum				
X	Colon				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals
X	Gallbladder	X	Urinary bladder		Lacrimal gland
X	Pancreas	XX	Testes	X	Mammary gland
		X	Epididymides	XX	Thyroids
		X	Prostate	XX	Parathyroids
		X	Seminal vesicle		
		XX	Ovaries		
	Respiratory	X	Ureters		Other
X	Trachea	X	Uterus	X	Bone (sternum and femur)
XX	Lung			X	Skeletal muscle
				X	Skin† (abdominal-untreated and top of head-untreated and unshaved)
				X	All gross lesions and masses

Results:

a. Organ Weights: There were no effects of dosing on organ weights. The only change was a significant ($p < 0.05$) increase in the absolute and relative weight of the right testis and a significant ($p < 0.05$) decrease in absolute and relative weights of the right ovary relative to the corresponding control. The author suggested that these changes are of no toxicological significance.

†Recommended by Subdivision F (November 1984) Guidelines.

b. Gross Pathology: Gross necropsy showed single or multiple scabs at the application site in 2/5 males and 2/5 females from control and treated groups. Other gross morphological changes included single or multiple, pale or depressed areas of the liver and/or dark areas of the lungs in the control and dosed animals. No other macroscopic changes were noted in control or treated groups.

c. Microscopic Pathology: Histopathological findings at the application site included hyperkeratosis, crusting, epidermal erosion/ulceration with hyperplasia, fibrosis, and/or cell infiltration in the upper dermis. Findings in the liver included necrosis, fibrosis, granulomatous inflammation, mononuclear or mixed cell infiltration, and/or vacuolation of hepatocytes. All findings were seen in dosed and control animals. Pathological changes in other tissues occurred randomly or were considered to be normal occurrences for the age and strain of the animal.

D. STUDY AUTHORS' CONCLUSIONS:

Mesurol solution in saline was applied to the shaved skin of male and female rabbits for 6 hours/day for 21 days to test the toxic and irritating effects of the compound. The control group consisted of saline-treated animals with equal numbers of male and female rabbits treated in a similar manner. Mesurol caused no mortality. The main toxicity of mesurol was seen as reduced food consumption and decline in body weight and body weight gain in female rabbits. No changes in hematology were found in either sex; the changes in SGOT and SGPT and calcium levels in treated animals were considered to be of no toxicological significance. Neither erythrocyte nor brain cholinesterase levels were changed in either sex of treated animals. Plasma cholinesterase activities were significantly reduced in dosed females relative to values of the controls at day 14 but were normal at study termination. All pretreatment levels for blood cholinesterase were relatively higher on the average than the controls in both sexes. Aside from transient clinical signs of toxicity found in two treated males due to accidental ingestion of mesurol on day 10 of the study, no significant gross or histopathological findings, or changes in organ weight, were observed.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The major deficiency in the design of the study was the use of only one dose level without justification. The EPA Pesticide Guidelines (1984) suggest the use of the "limit test" if that

dose is at least 1000 mg/kg body weight and if observable toxic effects are not produced; this study was conducted using 500 mg/kg body weight. In addition, the author did not provide individual animal data for the clinical signs of toxicity; therefore, the reviewers could not confirm the findings. Analyses methodology and results for compound purity, homogeneity, and stability were not presented, although the author indicated that mesurol was tested for all of the above. The methods of analysis for erythrocyte, plasma, and brain cholinesterase should be submitted for review.

The reviewers agree with the study author that there was no systemic toxicity aside from the clinical toxicity symptoms due to the two cases of accidental ingestion of mesurol. Although the author indicated that the minor signs of skin irritation observed in both control and treated animals were unrelated to the compound, it is suggested by the reviewers that a higher dose of the test material may result in skin irritation. The LOEL and NOEL values cannot be determined because only one dose was used. Additionally dates of individual back shaving must be submitted for evaluation. Buck 8/29/91

13544

048690

Chemical:

PC Code:
HED File Code
Memo Date: 09/13/91
File ID: 00000000
Accession Number: 000-05-0050

HED Records Reference Center
08/07/2002