007599

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

NOV - 2 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: REPEATED DOSE DERMAL TOXICITY:
21-DAY DERMAL STUDY WITH DPX-X1179-394
(METHOMYL) IN RABBITS

TO: JOANNE EDWARDS
PRODUCT MANAGER (74)
REGISTRATION DIVISION (H7505C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 10/24/89
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 10/26/89
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. M. van Gemert 10/30/89
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT: DUPONT & COMPANY
CHEMICAL: ETHANIMIDOTHIOIC ACID N-[[(METHYLAMINO)-CARBONYL]OXY]-, METHYL ESTER
SYNONYM: S-METHYL N-[METHYLCARBAMOYL)OXY]-THIOACETIMIDATE; METHOMYL
PROJECT: 9-2272
CASWELL NO.: 549C
RECORD NO.: 253298
IDENTIFYING NO.: 352-361
MRID NO.: 412515-01
ACTION REQUESTED: NONE SPECIFIED; 21-DAY DERMAL STUDY SUBMITTED IN RESPONSE TO METHOMYL REGISTRATION STANDARD REQUIREMENT.

COMMENT: THE REGISTRANT HAS SUBMITTED A 21-DAY DERMAL STUDY ON METHOMYL IN RESPONSE TO THE METHOMYL REGISTRATION STANDARD REQUIREMENT. THIS STUDY HAS BEEN REVIEWED, AND THE DER IS ATTACHED.

THE NOEL FOR SYSTEMIC TOXICITY CAN BE SET AT 5 MG/KG, AND THE LEL CAN BE SET AT 50 MG/KG, BASED ON CHOLINESTERASE INHIBITION (PLASMA AND BRAIN). NO DERMAL IRRITATION WAS OBSERVED.

REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 9/24/89
SECTION II, TOX. BRANCH II (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K. Clark Swentzel 10/26/89
HEAD SECTION II, TOX. BRANCH II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-DAY DERMAL TOXICITY - RABBITS TOX. CHEM. NO.: 549C

MRID NO.: 412515-01

TEST MATERIAL: ETHANIMIDOTHIOIC ACID N-[[(METHYLAMINO)-CARBONYL]OXY]-, METHYL ESTER

SYNONYMS: METHOMYL; S-METHYL N-[METHYLCARBAMOYL)OXY]-THIOACETIMIDATE; DPX-X1179-394

STUDY NUMBER: HLR 387-89

SPONSOR: DUPONT & COMPANY

TESTING FACILITY: HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

TITLE OF REPORT: REPEATED DOSE DERMAL TOXICITY: 21-DAY STUDY WITH DPX-X1179-394

AUTHORS: WILLIAM J. BROCK

REPORT ISSUED: AUGUST 29, 1989

CONCLUSIONS: RABBITS WERE DERMALLY EXPOSED TO DPX-X1179-394 FOR 21-DAYS AT DOSE LEVELS OF 0, 5, 50, AND 500 MG/KG/DAY. THE NOEL FOR SYSTEMIC TOXICITY CAN BE SET AT 5 MG/KG AND THE LEL AT 50 MG/KG, BASED ON CHOLINESTERASE INHIBITION (PLASMA AND BRAIN).

CLASSIFICATION: CORE MINIMUM.

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

A. MATERIALS:

1. TEST COMPOUND: METHOMYL; DESCRIPTION: WHITE SOLID; BATCH NO.: NOT PROVIDED- MEDICAL RESEARCH NO. 8698-001 AND HASKELL NO. 17,720; PURITY: 98.35%; STABILITY: ASSUMED STABLE BY STUDY DIRECTOR.

2. TEST ANIMAL: SPECIES: RABBIT; STRAIN: NEW ZEALAND WHITE; AGE: 8 WEEKS; WEIGHT: 1517-1985 G ON ARRIVAL, 2350-2450 G ON DAY 1; SOURCE: HARE MARLAND, HEWITT, NEW JERSEY.

3. STATISTICS: BODY WEIGHT, BODY-WEIGHT GAINS, AND ORGAN WEIGHTS: ANALYZED BY A ONE-WAY ANALYSIS OF VARIANCE; WHEN TEST FOR DIFFERENCES AMONG GROUP MEANS (F-TEST) WAS SIGNIFICANT FOR BODY WEIGHT AND BODY-WEIGHT GAINS, PAIRWISE COMPARISONS WERE MADE BETWEEN CONTROL AND TEST GROUPS WITH THE LEAST SIGNIFICANT DIFFERENCE (LSD) TEST. ORGAN WEIGHTS WERE ANALYZED BY FISHER'S EXACT TEST WITH A BONFERRONI CORRECTION. CLINICAL LABORATORY MEASUREMENTS: ANALYZED BY A ONE-WAY ANALYSIS OF VARIANCE AND BARTLETT'S TEST. WHEN THE F-TEST WAS SIGNIFICANT, COMPARISON WAS MADE WITH DUNNETT'S TEST. WHEN THE BARTLETT'S TEST WAS SIGNIFICANT, THE KRUSKAL-WALLIS AND MANN-WHITNEY U TEST WERE USED. TESTS FOR THE COMPARISON OF MEANS WERE CONSIDERED SIGNIFICANT AT THE ALPHA = 0.05.

B. STUDY DESIGN:

1. METHODOLOGY

RABBITS WERE ASSIGNED RANDOMLY (USING COMPUTER-RANDOMIZATION BASED ON BODY WEIGHT WITHIN THE SEX) TO THE FOLLOWING TEST GROUPS, BASED ON A RANGE-FINDING STUDY AND OTHER STUDIES ON METHOMYL.

TEST GROUP	TEST MATERIAL (MG/KG/DAY)	MALES	FEMALES
1	0 (DEIONIZED WATER)	10	10
2	5	5	5
3	50	5	5
4	500	10	10

EACH ANIMAL WAS CAGED INDIVIDUALLY AND HAD FREE ACCESS TO WATER AND FOOD (PURINA HIGH-FIBER RODENT CHOW 4®4 #5325). THE HAIR WAS CLOSELY CLIPPED FROM THE SCAPULAR TO THE LUMBAR REGION OF THE BACK OF EACH ANIMAL (EXPOSING APPROXIMATELY 190 CM^2 OF THE TOTAL BODY SURFACE AREA) ONE DAY PRIOR TO INITIAL EXPOSURE, AND EACH ANIMAL WAS FITTED WITH A PLASTIC COLLAR TO PREVENT INGESTION OF TEST MATERIAL AND DISRUPTION OF THE WRAPPINGS. THE TEST MATERIAL WAS MIXED WITH 5 ML DEIONIZED WATER AND QUANTATIVELY TRANSFERRED TO A 9" X 3" GAUZE PAD, WHICH WAS THEN PLACED ON THE SKIN. THE DOSING PREPARATIONS WERE PREPARED DAILY, EITHER AS A PASTE (HIGH-DOSE) OR SLURRY/SUSPENSION (LOW- & MID-DOSE). THE AUTHOR STATED THAT THE PROCEDURE ALLOWED FOR A UNIFORM DISTRIBUTION OF THE TEST MATERIAL ON THE SKIN. THE RABBITS WERE WRAPPED WITH SUCCESSIVE LAYERS OF PLASTIC FILM, STRETCH GAUZE BANDAGE, AND PLASTIC ADHESIVE BANDAGE AND RETURNED TO THEIR CAGES (INDIVIDUAL CAGES). THE TEST MATERIAL REMAINED IN PLACE FOR APPROXIMATELY 6 HOURS EACH

DAY FOR 21 DAYS. FOLLOWING EXPOSURE, THE WRAPPINGS WERE REMOVED, THE TREATED SKIN WAS WASHED GENTLY WITH WARM WATER AND PATTED DRY. CONTROL ANIMALS WERE TREATED SIMILARLY. DOSE LEVELS WERE BASED ON THE MOST RECENT BODY WEIGHT OF THE ANIMAL.

2. OBSERVATIONS

ALL RABBITS WERE EXAMINED DAILY FOR SIGNS OF ILL HEALTH, BEHAVIORAL CHANGES, OR OTHER SIGNS OF TOXICOSIS, AND TWICE DAILY FOR MORBIDITY AND MORTALITY. PRIOR TO EACH APPLICATION, ONE HOUR AFTER EXPOSURE, AND DAILY, THE SKIN OF EACH ANIMAL WAS GRADED ACCORDING TO THE DRAIZE SYSTEM. ALL ANIMALS WERE WEIGHED TWICE WEEKLY PRIOR TO EACH APPLICATION AND WEEKLY DURING THE RECOVERY PHASE. FOOD CONSUMPTION WAS MEASURED AT WEEKLY INTERVALS FOR EACH GROUP, AND INDIVIDUAL CONSUMPTION WAS CALCULATED.

RESULTS

SURVIVAL AND CLINICAL SIGNS

ONE HIGH-DOSE FEMALE WAS FOUND DEAD, DUE TO A FRACTURE AT THE THORACIC-CERVICAL JUNCTION OF THE VERTEBRAL COLUMN. ALL OTHER ANIMALS SURVIVED TO STUDY TERMINATION. THE HIGH-DOSE MALES DISPLAYED A GREATER INCIDENCE ($P<0.05$) OF HYPERREACTIVITY (INCREASED REACTION TO STIMULI-NOISE) COMPARED TO CONTROL, AND THE INCIDENCE IN HIGH-DOSE FEMALES WAS ALSO INCREASED BUT A $P<0.05$ WAS NOT ATTAINED. THE AUTHOR CONSIDERED THIS TO BE RELATED TO CHOLINESTERASE INHIBITION.

BODY WEIGHT AND FOOD CONSUMPTION

NO DIFFERENCES WERE REPORTED AMONG THE GROUPS WITH RESPECT TO BODY WEIGHT AND BODY-WEIGHT GAIN, AND FOOD CONSUMPTION AND FOOD EFFICIENCY WERE COMPARABLE AMONG THE GROUPS. THERE WAS A WIDE VARIABILITY IN WEIGHT GAIN, WITH STATISTICALLY SIGNIFICANT DIFFERENCES NOTED DURING DAYS 15-19 IN THE MID- AND HIGH-DOSE FEMALES (DECREASE; NOT DOSE-RELATED) AND IN THE LOW-DOSE MALES (INCREASE). THE ONLY OTHER STATISTICALLY SIGNIFICANT DIFFERENCE NOTED DURING THE DOSING PHASE WAS A DECREASE IN THE LOW-DOSE MALES DURING DAYS 12-15.

DERMAL IRRITATION

THERE WAS NO INCREASE IN DERMAL IRRITATION THAT COULD BE RELATED TO TREATMENT.

3. CLINICAL PATHOLOGY

BLOOD WAS COLLECTED FROM EACH RABBIT FOR HEMATOLOGY AND CLINICAL ANALYSES APPROXIMATELY 1 HOUR FOLLOWING THE LAST TREATMENT (AFTER UNWRAPPING) FROM 5 RABBITS/SEX/DOSE AND FROM THE REMAINING 5/SEX FOR THE CONTROL AND HIGH-DOSE GROUPS AFTER THE 14-DAY RECOVERY PERIOD. THE CHECKED (X) PARAMETERS WERE EXAMINED.

007599

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 CONC. (MCHC)
X	ERYTHROCYTE COUNT (RBC)	X	MEAN CORPUSCULAR VOLUME (MCV)
X	PLATELET COUNT		NUCLEATED RED BLOOD CELL COUNT
	HEINZ BODIES (HzB)		CELLULAR MORPHOLOGY
	METHEMOGLOBIN (METHB)		RETICULOCYTE COUNT

RESULTS

STATISTICALLY SIGNIFICANT DIFFERENCES WERE OBSERVED AS FOLLOWS.

	21-DAY		RECOVERY	
	MALES	FEMALES	MALES	FEMALES
MCV	-	-	-	HD INC
MCH	-	-	-	HD INC
MCHC	-	-	HD INC	HD INC
PLAT	-	MD DEC	-	-

INC = INCREASE, DEC = DECREASE, MD = MID DOSE, HD = HIGH DOSE, - = NO EFECT

B. CLINICAL CHEMISTRY

ELECTROLYTES:

X	CALCIUM
X	CHLORIDE
	MAGNESIUM
X	PHOSPHOROUS
X	POTASSIUM
X	SODIUM

ENZYMES

X	ALKALINE PHOSPHATASE
	CHOLINESTERASE
	CREATININE PHOSPHOKINASE
	LACTIC ACID DEHYDROGENASE
X	SERUM ALANINE AMINOTRANSFERASE (ALSO SGPT)
X	SERUM ASPARTATE AMINOTRANSFERASE (ALSO SGOT)
	GAMMA GLUTAMYL TRANSFERASE
	GLUTAMATE DEHYDROGENASE

OTHER:

X	ALBUMIN
X	BLOOD CREATININE
X	BLOOD UREA NITROGEN
X	CHOLESTEROL
X	GLOBULINS
X	GLUCOSE
X	TOTAL BILIRUBIN
X	TOTAL SERUM PROTEIN
	TRIGLYCERIDES
	SERUM PROTEIN ELECTROPHORESIS
	ALBUMIN/GLOBULIN RATIO

RESULTS

THERE WAS AN INCREASE IN GLUCOSE IN THE LOW- AND HIGH-DOSE MALES ON DAY 21 ONLY. HIGH-DOSE FEMALES DISPLAYED AN INCREASE IN ALT LEVELS FOLLOWING RECOVERY.

C. CHOLINESTERASE ACTIVITY

WHOLE BLOOD AND PLASMA CHOLINESTERASE ACTIVITIES WERE DETERMINED. RED BLOOD CELL CHOLINESTERASE ACTIVITY WAS CALCULATED FROM THE PLASMA AND WHOLE BLOOD CHOLINESTERASE RESULTS AND HEMATOCRIT. BRAIN CHOLINESTERASE WAS MEASURED AT STUDY TERMINATION.

RESULTS

AT DAY 21, MID- AND HIGH-DOSE MALES AND HIGH-DOSE FEMALES DISPLAYED SIGNIFICANTLY LOWER PLASMA CHOLINESTERASE ACTIVITIES. MEAN RBC CHOLINESTERASE ACTIVITY WAS ALSO DECREASED IN THE HIGH DOSE GROUPS, BUT ONLY SLIGHTLY. BRAIN CHOLINESTERASE ACTIVITY WAS SIGNIFICANTLY DECREASED IN BOTH SEXES OF THE HIGH-DOSE. FOLLOWING RECOVERY, NO DIFFERENCES WERE OBSERVED BETWEEN CONTROL AND HIGH-DOSE ANIMALS IN CHOLINESTERASE ACTIVITIES.

	PLASMA ChE	RBC ChE	BRAIN ChE
MALES			
DAY 21			
CONTROL	482	2553	6.83
LOW	483	2690	6.38
MID	369*	2446	6.18
HIGH	176*	2050	3.30*
AFTER RECOVERY			
CONTROL	497	2590	7.05
LOW	-	-	-
MID	-	-	-
HIGH	441	2673	6.14

	PLASMA ChE	RBC ChE	BRAIN ChE
FEMALES			
DAY 21			
CONTROL	461	2801	6.91
LOW	489	3350	6.98
MID	413	2879	5.95
HIGH	252*	2380	4.73*
AFTER RECOVERY			
CONTROL	479	2572	6.47
LOW	-	-	-
MID	-	-	-
HIGH	414	2916	6.05

*$P < 0.05$

4. GROSS PATHOLOGY

ALL ANIMALS OF THE LOW- AND MID-DOSE GROUPS AND 5/SEX OF THE HIGH-DOSE AND CONTROL GROUPS WERE SACRIFICED FOLLOWING BLOOD COLLECTION ON THE FINAL TREATMENT DAY AND WERE SUBJECTED TO GROSS PATHOLOGICAL EXAMINATION. SURVIVORS OF THE CONTROL AND HIGH-DOSE GROUPS WERE SACRIFICED 14 DAYS AFTER THE LAST TREATMENT AND NECROPSIED. THE LIVER, KIDNEYS, ADRENALS, SPLEEN, BRAIN, AND TESTES WERE WEIGHED AND RELATIVE ORGAN WEIGHTS WERE CALCULATED.

RESULTS

THE MEAN ABSOLUTE BRAIN WEIGHT OF THE LOW-DOSE MALES WAS SIGNIFICANTLY LOWER THAN CONTROL VALUES FOLLOWING SACRIFICE AT DAY 21. FOLLOWING THE RECOVERY PHASE, MEAN ABSOLUTE AND RELATIVE SPLEEN WEIGHTS OF THE HIGH-DOSE GROUP WERE SIGNIFICANTLY BELOW CONTROL VALUES. THERE WERE NO HISTOPATHOLOGICAL CHANGES THAT CORRELATED WITH THESE WEIGHT DIFFERENCES.

5. HISTOPATHOLOGY

THE FOLLOWING ORGANS AND TISSUES WERE PRESERVED FROM ALL RABBITS, AND FURTHER PROCESSED TO SLIDES AND EXAMINED MICROSCOPICALLY FROM THE CONTROL AND HIGH-DOSE ANIMALS (INCLUDING THE ANIMAL THAT DIED ON TEST). THE LIVER, KIDNEYS, BRAIN, TREATED AND UNTREATED DORSAL SKIN, AND GROSS LESIONS FROM THE LOW- AND MID-DOSE RABBITS WERE ALSO EXAMINED.

DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**	
	TONGUE	X	AORTA	X	BRAIN
X	SALIVARY GLANDS	X	HEART	X	PERIPH. NERVE (SCIATIC)
X	ESOPHAGUS	X	BONE MARROW	X	SPINAL CORD
X	STOMACH	X	LYMPH NODES*	X	PITUITARY
X	DUODENUM	X	SPLEEN†	X	EYES
X	JEJUNUM	X	THYMUS	**GLANDULAR**	
X	ILEUM	**UROGENITAL**		X	ADRENALS
X	CECUM	X	KIDNEYS†		LACRIMAL GLAND (HARDERIAN)
X	COLON	X	URINARY BLADDER		MAMMARY GLAND
X	RECTUM	X	TESTES	X	PARATHYROIDS
X	LIVER†	X	EPIDIDYMIDES	X	THYROIDS
X	GALL BLADDER	X	PROSTATE	**OTHER**	
X	PANCREAS		SEMINAL VESICLE	X	BONE (STERNUM)
RESPIRATORY		X	OVARIES	X	SKELETAL MUSCLE
X	TRACHEA	X	UTERUS	X	SKIN (TREATED AND UNTREATED)†
X	LUNG		CERVIX	X	ALL GROSS LESIONS AND MASSES†
	NOSE	X	VAGINA		HEAD
	PHARYNX		OVIDUCT		COAGULATING GLAND
	LARYNX				MEDIASTINAL/MESENTERIC TISSUE

* MANDIBULAR AND MESENTERIC
†INTERMEDIATE AND LOW DOSE GROUPS

RESULTS

NO DIFFERENCES WERE OBSERVED BETWEEN THE CONTROL AND HIGH-DOSE ANIMALS.

C. CONCLUSION

THE NUEL FOR SYSTEMIC TOXICITY CAN BE SET AT 5 MG/KG, AND THE LEL CAN BE SET AT 50 MG/KG, BASED ON CHOLINESTERASE INHIBITION (PLASMA AND BRAIN). NO DERMAL IRRITATION WAS OBSERVED.