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

MAR 24 1998

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of toxicity studies on DDVP using human volunteers
PC Code 084001 DP Barcode 235158 Tox Chem No 328
MRID Nos 44317901, 4416201, 44248801, 44248802

From: Joycelyn E. Stewart, Ph.D., Pharmacologist,
Registration Action Branch II (7509C) 3/9/98

Thru: Richard Loranger, Ph.D., Senior Scientist,
Registration Action Branch II R. Loranger 3/10/98

To: Christina Scheltema, Chemical Manager,
Risk Characterization and Analysis Branch

Registrant: Amvac Chemical Corporation
Commerce, California

Action Requested: Review human studies submitted in response to publication of Agency PD2/3 on DDVP.

Conclusion: RAB II has reviewed the subject studies and has concluded as follows:

The three studies submitted can be classified Acceptable-non Guideline. They were not intended to fulfill any Guideline requirements. For your convenience, Executive Summaries and DER's of the studies are attached.

1. A Study to investigate erythrocyte cholinesterase inhibition following oral administration to healthy male volunteers.

EXECUTIVE SUMMARY: Fasted Caucasion male subjects were administered a single oral dose of 35 mg DDVP in corn oil, followed by a placebo dose of corn oil capsules , then a second dose of 35 mg of DDVP in corn oil (Phase I). All doses were in a volume of 0.5 mL. Prior to dosing, three baseline cholinesterase measurements were taken, and volunteers were given a thorough medical examination. A symptom form was kept for each individual to record any adverse physical signs. RBC cholinesterase activity was monitored immediately prior to dosing,

and on study days 1, 3, 5/6, and 7. Under the study conditions, RBC cholinesterase activity was not inhibited. The NOEL was 35 mg, equivalent to 0.5 mg/kg. When the same volunteers were administered 21 mg of DDVP daily for 12 or 15 days (Phase 2), and RBC cholinesterase activity monitored on days 3, 5, 8, 10, 12, 15, 19, 22, and 24 after dosing and additional sampling done on days 26, 29, 33, 40, 47, and 54, this activity was statistically significantly and persistently reduced. A NOEL was not established for this phase. The LOEL was 21 mg, equivalent to 0.3 mg/kg/day.

2. Dichlorvos: A single blind, placebo controlled, randomized study to investigate the effects of multiple oral dosing on erythrocyte cholinesterase inhibition in health male volunteers.

EXECUTIVE SUMMARY: In a single blind oral study, fasted male volunteers were administered DDVP in capsules at a dose of 7 mg (equivalent to approximately 0.1 mg/kg/day) in corn oil for 21 days. Control subjects received corn oil as a placebo. Any adverse events suffered by the participants were recorded on adverse events forms. Baseline values for RBC cholinesterase activity for each study participant was determined on days -14, 12, -10, -7, -5, -3, and immediately prior to dosing, and RBC cholinesterase activity was monitored on days 2, 4, 7, 9, 11, 14, and 18.

No toxicity was reported which could be attributed to DDVP administration. While there were significant decrements in RBC cholinesterase activity at some reporting periods, the overall mean did not exceed 16 percent at any time. The LOEL for RBC cholinesterase inhibition is 0.1 mg/kg/day. A NOEL is not established.

3. Dichlorvos: A study to investigate the effects of a single oral dose on erythrocyte cholinesterase inhibition in healthy male volunteers.

EXECUTIVE SUMMARY: Dichlorvos was administered in a single oral dose of 70 mg (equivalent to 1 mg/kg) to fasted young healthy male volunteers. Prior to dosing, baseline RBC cholinesterase activity was measured on study days -22, -20, -18, -15, -13, -11, -8, -6, -4, and immediately prior to dosing. The study subjects were medically supervised for clinical signs and body temperature changes for 24 hours and for RBC cholinesterase inhibition for up to 14 days post compound administration.

Under the study conditions, no adverse clinical signs and no body temperature variations were reported. Mean RBC cholinesterase activity was statistically significantly inhibited on days 5/6, 7, and 14, but the percent decrement was 12 percent or less.. No reduction in RBC cholinesterase activity was apparent at the other reporting periods. The reduction in cholinesterase activity was considered biologically meaningful. Under the study conditions, the LOEL is 1 mg/kg. A NOEL was not established.

1Reviewed by: Joycelyn E. Stewart, Ph.D. 2/11/98 012541
Registration Action Branch II (H7509C)
Secondary Reviewer: William Sette, Ph.D. 2/12/98
Scientific Analysis Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute and Subchronic/ Human volunteers

TOX. CHEM. No.: 328

MRID No.: 44317901

GUIDELINE #: Non-guideline

TEST MATERIAL: 2,2,dichlorovinyl dimethyl phosphate

SYNONYMS: Dichlorvos, DDVP, Dedevap, Vapona

STUDY NUMBERS: XH 5170

SPONSOR: Amvac Chemical Corporation

TESTING FACILITY: Central Toxicology Laboratory, Adderly Park, Macclesfield, Cheshire, U.K.

TITLE OF REPORT: Dichlorvos: A study to investigate erythrocyte cholinesterase inhibition following oral administration to healthy male volunteers.

AUTHORS: Miss A.J. Gledhill

REPORT ISSUED: 3 February, 1997

EXECUTIVE SUMMARY: Fasted Caucasian male subjects were administered a single oral dose of 35 mg DDVP, followed by a placebo dose of corn oil capsules then a second dose of 35 mg DDVP (Phase 1). All doses were in a volume of 0.5mL. Prior to dosing, three baseline cholinesterase measurements were taken, and volunteers were given thorough medical examinations. A symptom form was kept for each volunteer to record any adverse physical signs. RBC cholinesterase activity was monitored immediately prior to dosing, and on study days 1, 3, 5/6, and 7. Under the study conditions, RBC cholinesterase was not inhibited in phase I. The NOEL was 35 mg, equivalent to 0.5 mg/kg based on the absence of reduction of cholinesterase activity. When the same volunteers were administered 21 mg of DDVP daily for twelve or 15 days (Phase 2), the NOEL was < 21 mg, equivalent to 0.3 mg/kg/day, based on sigificant and persistent reduction of cholinesterase activity. This is a non-guideline study.

MATERIALS: Dichlorvos (lot number 402010A) 98% pure was the test chemical. Corn oil Y09341/001 was the vehicle. Red size 0 gelatin capsules were supplied by Zeneca Pharmaceuticals. Six healthy male volunteers age 21-30 years, weighing 67-80 kg were the test subjects.

METHODS:

1. Dosing preparation

For the 35 mg dose dichlorvos was dissolved at a concentration of 70 mg/mL, and for the 21 mg dose it was dissolved at a concentration of 42 mg/mL in corn oil. In order to ensure that the correct compound was prepared at the required concentration two staff persons were always present during the preparation of the dose formulation and the dosing capsules. The dose formulations were first passed through a sterile filter into a sterile container, then placed in sterile # 0 capsules which had previously been examined for Salmonella, Listeria, and general aerobic organisms such as Streptococcus and Staphylococcus. Capsules were stored individually in sterile containers. Dosing volume was 500 µL. The dichlorvos content of the dosing formulations (both before and after capsule preparation) were analysed by gas chromatography using a Hewlett Packard HP589A gas chromatograph using an electron capture detector and calibrated against a standard curve.

2. Study Design

Prior to study start, and at study termination, each participant was given a thorough medical history review and physical examination. Blood was withdrawn for standard hematology and biochemistry, determination of hepatitis B antigen, and human immunodeficiency virus. Heart rate, blood pressure, and EKG were measured. Urine was collected for standard urinalysis and a drugs of abuse screen. In addition, a drugs of abuse screen was conducted just prior to study start. Prior to study start, on days -7, -5, -3, blood was withdrawn for cholinesterase determination for each individual in order to determine baseline values. The study was conducted in two phases. Phase I consisted of three parts. In the first part, the study subjects were fasted, then administered DDVP 35 mg (equivalent to 0.5 mg/kg) in corn oil in gelatin capsules on dosing day 1. In part 2, study subjects received corn oil in gelatin capsules, and in part 3 they received 35 mg of DDVP in corn oil. In each section of the study, the study subjects reported to the testing unit the evening prior to dosing and remained in the unit overnight after being adminstered the chemical. Participants were allowed one to two days in between each section of the study when they were not administered either DDVP or the placebo. RBC cholinesterase activity was monitored at 24, 72, 120, and 168 hours post dose in part 1; 24, 72, and 120 hours post dose in part 2, and at 24, 72, 120, and 168 hours post dose in Part 3. Body temperature was recorded pre-dose and at 2, 4, 8, 12, and 24 hours after dosing.

In Phase 2, the same fasted participants each received 21 mg (equivalent to 0.3 mg/kg) of dichlorvos in corn oil in gelatin capsules taken in 150 mL of distilled water for 12 or 14 days. Blood was collected for cholinesterase activity determinations on days 3, 5, 8, 10, 12, 15, 17, 19, 22, and 24 after dosing, and additional samples were collected on days 26, 29, 33, 40, 47 and 54 after the first dose to further monitor RBC cholinesterase activity. If RBC cholinesterase activity fell during Phase I and did not return to within 15% of the lowest pre-dose value the interval between Phases I and 2 would be reviewed and extended if necessary. If RBC cholinesterase activity decreased to 80 percent or less of the lowest pre-dose value followed by a further decrease in the next successive sample, the participant would be removed from the study.

Volunteers remained overnight in the testing facility for 24 hours post compound administration under constant medical supervision. Any clinical signs observed were reported on adverse events forms by the study investigator. Urine samples were collected for clinical and pharmacokinetic evaluation.

For cholinesterase activity testing, 10 mL of blood was withdrawn into heparinized tubes, centrifuged at 2000 rpm at ambient temperature for 15 minutes to separate plasma and red blood cells. The red blood cells were transported in insulated containers to CTL immediately after the plasma had been removed. Cholinesterase determinations were done immediately upon the receipt of the samples, using a modified Ellman method with a Kone Specifc analyzer. Plasma was snap frozen using dry ice and acetone and stored in polypropylene screw top vials at -20° C.

Clinical procedures were performed at Medeval Limited, University of Manchester, U.K. Laboratory determinations were done at Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, U.K.

3. Study Inclusion

To be included in the study volunteers had to meet the following criteria: be between the ages of 18 and 45 and of a single ethnic group; have normal clinical examination including history and EKG; clinical chemistry, hematology, and urinalysis within the normal range; RBC cholinesterase activity above 10000 IU/L: negative screen for serum hepatitis B and HIV; negative screen for drugs of abuse; weigh between 60 and 90 kg, and not vary by more than 15% from the desirable weight as described by Metropolitan Life Tables.

4. Restrictions on Study participants

Study participants were required to do the following: avoid exposure to organophosphate compounds during and for two months after the study: abstain from taking medication from 24 hours before or until 48 hours after dosing; abstain from driving or

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; abstain from driving or operating machinery on the dosing day; abstain from smoking, caffeine containing drinks before each dosing day and until 24 hours after dosing; abstain from alcohol for 24 hours before and for 5 days after dosing; abstain from strenuous physical activity, including sports for 72 hours before dosing until 72 hours after dosing; abstain from potential hazardous activity for 48 hours after dosing; fast from 12 midnight on the night before dosing; remain in the testing unit for 24 hours after dosing. Participants were free to withdraw from the study at any time.

PROTECTION OF STUDY SUBJECTS: The study was conducted in accordance with the Declaration of Helsinki including all amendments up to and including the Hong Kong revision. The study protocol was approved by the Medeval Independent Ethics Committee, University of Manchester, U.K.

QUALITY ASSURANCE: Signed and dated quality assurance statements were included with the submission.

STATISTICAL ANALYSIS: Differences from pre-dose averages were analysed by comparing post-dose group means for each time point and each phase using a paired t-test(SAS, 1989)

Differences between pre-study and subsequent cholinesterase levels for each individual at each time point were compared using the Goode permutation test.

RESULTS:

Analysis of the dosage formulations as well as the test capsules demonstrated that the doses achieved were within ± 12 percent of target concentration.

In phase I, part 1, no changes in body temperature were reported for any of the volunteers administered DDVP. One study subject reported some drowsiness, and one reported a slight headache, none of which were attributed to administration of the chemical, though no reasons were given to support these judgements. After day 1 dosing, group mean erythrocyte cholinesterase levels were 93% of pre-dose values, and were 100, 90, and 101 percent of predose values on days 3, 5, and 7 respectively.

In phase I, part 2, none of the volunteers receiving the placebo demonstrated any changes in body temperature. One volunteer reported a slight headache, while one reported some drowsiness. RBC cholinesterase activity in these volunteers were not reduced from their pre-dose values.

In phase 1 part 3, all of the volunteers received the second dose of dichlorvos. No symptoms attributable to administration of dichlorvos were reported. Group mean RBC cholinesterase activity after administration of DDVP range between 94 and 98 percent of

predose activity. Individual post-dose cholinesterase activity ranged from 85 to 100 percent of pre-dose values at all reporting periods as shown in Table I, taken from the investigators' report.

In phase 1, no symptoms were reported when DDVP was administered that were not reported when the placebo was administered.

In phase 2, two study subjects did not report any symptoms. One volunteer had an upper respiratory tract infection between days 7 and 12, one reported feeling tired on days 5-9, with headache and nausea on day 6, one reported feeling anxious for an hour after the first dose, and one had some abdominal colic on day 12. The investigators did not attribute these symptoms to dichlorvos administration.

Group mean RBC cholinesterase activity was significantly decreased ($p<0.01$) when compared to the group mean pretest value from day 5 thru day 33. RBC cholinesterase activity decreased at each reporting period until day 22 when it started to recover. On day 22, the activity was 69 percent of control, after which it continued to recover until on day 54, when the final assessment was done, RBC cholinesterase had recovered to 91 percent of control. Individual RBC cholinesterase activity appeared to be more significantly reduced in the subjects who had not participaaaated in the first phase of the study, but all volunteers had significant RBC cholinesterase activity reduction at some reporting period. Cholinesterase activity for phase 2 is shown in Table 2, excerpted from the investigators' report.

Table 1. RBC Cholinesterase Activity (I.U.) in Human Volunteers Administered Oral Doses of Dichlorvos (35 mg) and Corn Oil (0.5 ml) in Phase I

Time	Subjects					
	I	II	III	IV	V*	VI*
Pre-Test (Mean)	16483	15460	16570	19910	18097	14993
Day 1 35 mg DDVP	15900 (96%)	13830 (89%)	14790 (89%)	15870 (79%)		
Day 3	16470 (100%)	15500 (100%)	16360 (99%)	20140 (101%)		
Day 5/6	14420 (87%)	14670 (95%)	14220 (86%)	18560 (93%)		
Day 7	15960 (96%)	16200 (100%)	16110 (100%)	20520 (103%)		
Corn Oil 0.5 ml Day 1	15330 (93%)	17300 (112%)	16700 (101%)	20160 (101%)		
Day 3	15010 (91%)	15780 (102%)	15040 (91%)	19650 (100%)		
Day 5/6	17860 (100%)	16340 (100%)	17180 (103%)	20490 (102%)		
DDVP 35 mg Day 1	15090 (91%)	15560 (100%)	16100 (100%)	18820 (94%)	16790 (92%)	15420 (102%)
Day 3	15170 (92%)	15410 (91%)	16120 (97%)	17870 (89%)	17170 (94%)	14760 (98%)
Day 5/6	16010 (97%)	15480 (100%)	15200 (91%)	17930 (90%)	15530 (85%)	15190 (101%)
Day 7	16270 (98%)	16070 (100%)	16140 (97%)	19340 (97%)	16560 (91%)	14780 (98%)

( ) indicates percent of individual RBC ChE activity as compared to the individual's mean predose ChE's value.
* Volunteers received only the second DDVP dose.

Table 2	Mean RBC cholinesterase activity in male volunteers administered DDVP (35 mg) in Phase I				
Treat-ment	Pre-Dose	Day 1	Day 3	Day 5	Day 7
DDVP 35 mg/kg	17106± 1936	15908± 990	17118± 2061	15468*± 2217	17198± 2217
% of mean	100	93	100	90	101
corn oil	17106± 1936	17373± 2033	16370± 2215	17698*± 1793	
% of mean	100	102	96	105	

Note: for the placebo treated subjects, cholinesterase activity was measured only up to day 5

n = 4

Table 2a	Mean RBC Cholinesterase Activity in Male Volunteers Administered a single oral dose of DDVP (35 mg) in Phase I				
Treat-ment	Pre-dose	Day 1	Day 3	Day 5	Day 7
DDVP	16919± 1816	16297± 1373	16083*± 1218	15890± 1043	16527± 1509
.	100	96	95	94	98

* Statistically significantly different from control
n = 6

Table 3. RBC Cholinesterase Activity (I.U.) in Human Volunteers Administered Oral Doses of Dichlorvos (21 mg) and Corn Oil in Phase II

Timepoint	Mean	SD	n	% of mean
predose	16919	1815.57	6	100
day 3	15260	1808.90	6	90
day 5	14425**	1676.99	6	85
day 8	13191.67**	777.16	6	78
day 10	13068.33**	1269.57	6	77

day 12	12968.00**	1873.17	6	77
day 15	12420.00**	1286.84	6	73
day 17	12035.00**	1545.86	6	71
day 19	12473.33**	1322.14	6	74
day 22	11675.00**	1042.26	6	69
day 24	12211.67**	1705.75	6	72
day 26	12402.00**	2197.96	5	73
day 29	12504.00**	1366.58	5	74
day 33	13634.00**	1467.93	5	81
day 40	13810.00*	994.13	3	82
day 55	15456.00*	1419.61	5	91

DISCUSSION:

As shown in Table 1, in Phase 1, the lowest percent RBC cholinesterase activity was demonstrated in Study Subject #4 on day one post dosing. However by day 3, the RBC cholinesterase activity had rebounded to 101 pecent of control. For all other study subjects, the RBC cholinesterase activity ranged from 86 to 103 percent during the observation periods. The mean day values for RBc cholinesterase activity was statistically significantly decreased for DDVP treated subjects when compared to controls. However, this value was 90 percent of the mean predose values. The values for RBC cholinesterase activity was also statistically significant lower on day 3 for the second DDVP dose. This mean value also was 95 percent of the predose maean. Based on these results, it is this reviewer's opinion that the RBC cholinesterase reductions were not biologically significant. It should also be noted DDVP is rapidly metabolized and excreted. In a rat metabolism study (MRID 41228701) in which rats were administered 1 mg or 20 mg ^{14}C DDVP/kg, 43 to 57 percent of the ^{14}C dose was eliminated in the urine, feces, and expired air within 24 hours after dosing.

The data presented demonstrate that under the study conditions, administration of a single oral dose of 35 mg dichlorvos did not result in any decrease of RBC cholinesterase activity in human volunteers after up to 7 days of monitoring. This was apparent both in the volunteers who received dichlorvos only, and in those who received dichlorvos followed by corn oil, then received dichlorvos again. The NOEL for phase I of the study is 35 mg of dichlorvos, equivalent to 0.5 mg/kg of dichlorvos. Although the investigators compared group means for significance, this reviewer determined that comparing the individual post dose cholinesterase activity values with each study participant's pre-dose mean activity was a more meaningful comparison of the data,

as shown in Table 1.

With respect to Phase 2 of the study, RBC cholinesterase inhibition started on day 10 and continued until day 24, when recovery began. This was apparent both in the group and the individual results (up to 31%). Consequently, a NOEL has not been established for this portion of the study.

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Reviewed by: Joycelyn E. Stewart, Ph.D. 7/21/98
Registration Action Branch (H7509C)
Secondary Reviewer: William Sette, Ph.D. for 7/21/98
Science Analysis Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic oral/human volunteers

TOX. CHEM. No.: 328

MRID No.: 442488-01

GUIDELINE #: Non-guideline

TEST MATERIAL: 2,2 dichlorovinyl dimethyl phosphate

SYNONYMS: dichlorvos, DDVP, dedevap, Vapona

STUDY NUMBERS: XH6063

SPONSOR: Amvac Chemical Corporation

TESTING FACILITY: Central Toxicology Laboratory
Adderly Park, Macclesfield,
Cheshire, U.K.

TITLE OF REPORT: Dichlorvos: A Single Blind, Placebo Controlled, Randomized Study to Investigate the Effects of Multiple Oral Dosing on Erythrocyte Cholinesterase Inhibition in Healthy Male Volunteers.

AUTHORS: Miss A.J. Gledhill

REPORT ISSUED: March 24, 1997

EXECUTIVE SUMMARY: In a single blind oral study fasted male volunteers were administered DDVP in capsules daily at a dosage of 7 mg (equivalent to approximately 0.1 mg/kg/day) in corn oil for 21 days. Control subjects received corn oil as a placebo. Any adverse events suffered by the participants were recorded on adverse events forms. Baseline values for RBC cholinesterase activity for each study participant were determined on days -14, -12, -10, -7, -5, -3, and immediately prior to dosing, and RBC cholinesterase activity was monitored on days 2, 4, 7, 9, 11, 14, 16, and 18.

No toxicity was reported which could be attributed to DDVP administration. While there were significant decrements in RBC cholinesterase activity in DDVP treated subjects at some reporting periods, the overall mean reduction from pretreatment values did not exceed 16 percent at any time. The cholinesterase activity values used to calculate the individual means varied by

up to 21 percent. **The LOEL for RBC cholinesterase inhibition is 0.1 mg/kg/day. The study is classified Acceptable-Non Guideline.**

MATERIALS:

Dichlorvos (lot # 608002S074), 98% a.i., described as a clear colorless liquid, was the test chemical. Corn oil (CTL reference # 00790/007/007, was the vehicle. Nine healthy Caucasion male volunteers, age 19-34 years, weighing 61 to 90 kg were the test subjects. In order to be included in the study, participants had to be between the ages of 18 and 45 years old, of a single ethnic group, be within normal range with respect to history and physical examinations, and EKG, routine clinical chemistry, hematology, urinalysis; have RBC cholinesterase values of 10,000 IU/L, be negative for hepatitis B antigen and the human immunodeficiency virus, and negative screen for drugs of abuse.

METHODS:

1. Dosing Preparation

Dichlorvos was dissolved in corn oil at 14 mg/mL, placed in capsules which were passed through a disposable sterile filter (0.2 μm Sartorius V115 filtraton unit) into a sterile container, then dispensed into sterile red # 0 gelatin capsules. Capsules were stored individually in sterile screw top containers. Two staff members were always present during the preparation of the dose formulations and dosing capsules in order to ensure accuracy in compound and dose preparation. Capsules were prepared on a weekly basis. The dichlorvos content of the dosing formulations, the dosing capsules and the stability of dichlorvos in the dosing formulation and capsules were determined by gas chromatography. Dosing volume was 0.5 mL.

2. Study Design

The study was a single blind, randomized, placebo controlled study. Nine healthy males were selected from the Medeval Volunteer Panel. Each study participant was given a complete medical history review and was given a thorough physical examination. Blood was drawn for standard hematology and biochemistry, determination of hepatitis B antigen, humanimmunodeficiency virus, and erythrocyte cholinesterase activity. Heart rate, blood pressure and EKG were measured, and urine was collected for standard urinalysis and a drugs of abuse screen.

On the day of dosing, participants were fasted from midnight then randomly assigned to treatment groups receiving either 1 mg/kg of dichlorvos in corn oil or the corn oil vehicle administered in gelatin capsules for twenty one days. Dosing volume was 500 μL. Each capsule was administered with 150 ml of water.

RBC cholinesterase activity was measured on study days -14, -12,

-10, -7, -5, -3, and immediately pre-compound administration to establish baseline cholinesterase activity. Following compound administration RBC cholinesterase activity was measured on days 1, 2, 4, 7, 9, 11, 14, 16, 18, 25, 28, and 29/30. Additional samples were taken from volunteers 2, 3, 5, and 6 on day 24 for determination of cholinesterase activity.

Blood was collected into heparinized tubes and centrifuged at 2000 rpm at ambient temperature for 15 minutes to separate plasma from red blood cells. Plasma was snap frozen using dry ice and acetone and stored at -20°C for possible additional study. RBC were analysed for cholinesterase using a modified Ellman method and a Kone Specific analyser (specifications to follow)

3. Protection of Subjects

The study was conducted in accordance with the Declaration of Helsiinki including all amendments up to and including the Hong Kong revision.

4. Exclusion fron the Study

The following persons were excluded from participation in the study: persons with frequent or regular use of medication or therapy; participation in another study within three months before the start of the study; any acute illness within two weeks of the start of the study; persons with suspected or definite personal or family history of adverse drug reactions or hypersensitivity to chemicals with a structure similar to that of dichlorvos; persons with history of g.i., hepatic or renal disease; donation of more than 1500 mL of blood in the 12 month period up to the end of the study; excessive alcohol intake; treatment within the previous three months with any drug known to have a well defined potential of toxicity to a major organ; persons exposed to organophosphorus compounds within the last three months; smokers of over 10 cigarettes per day; persons with planned need for general anesthesia within two months of the last day of study participation; and volunteers who, in the opinion of their physician should not participate in the study;.

QUALITY ASSURANCE: Signed and dated quality assurance statements were included in the submission.

STATISTICAL ANALYSIS: Differences in group mean cholinesterase activity between control and dichlorvos treated individuals at each time point were analysed by repeated measures analysis of variance using the MIXED procedure in SAS. The Leased-Squares Mean (LSM) for each period and treatment group were calculated, and the differences between the dichlorvos treated group LSM and the placebo treated group LSM were compared using a two-sided Student's t test based on the standard error in the analysis.

Differences between pre-study and subsequent group means for each treatment at each time point were compared using a paired t-test.

Differences between pre-study and subsequent cholinesterase levels for each individual were compared using the Good permutation test.

RESULTS: Several study participants reported non-specific symptoms but there were no sympatomatology which could be attributed to DDVP administration. Of the three control subjects, one (volunteer 1) reported mild dizziness on day 1, and coughing days 10 to 16; one (volunteer 5) reported forgetfulness and tiredness on days 3-7, and flatulence on days 16-18, and backache days 4 through the end of the study. This subject was administered analgesics by the study nurse on days 9 and 10. Of the six participants treated with DDVP, one (volunteer 8) complained of mild headache, one (volunteer 6) suffered a nosebleed, one (volunteer 2) reported tiredness on days 0-4, and one (volunteer 4) complained of nausea on day 1 and tiredness on days 1-22. The symptoms reported did not seem to be associated with the results of the RBC cholinesterase measurements.

The results of the RBC Cholinesterase activity, taken from the investigators' report, are shown in the following two tables

Table 1. Mean RBC Cholinesterase Activity (I.U.) in Male volunteers administered 7 mg DDVP (0.1 mg/kg/day) for 21 days

Time	placebo treated n = 3		DDVP treated n = 6	
	Mean ± S.D	%	Mean ± S.D.	%
Pre-dose	18484± 1347	100	17738± 1713	100
Day 1	17930± 1404	97	17628± 1914	99
Day 2	18180± 1565	98	16817*± 1547	95
Day 4	18740± 1771	101	16933**± 1598	95
Day 7	18530± 1888	100	16182± 1796	91
Day 9	18460± 1007	100	16708± 2505	94
Day 11	19210± 1036	104	16037± **1654	90
Day 14	18490± 1642	100	15333**± 1250	86
Day 16	17706± 2470	96	15912**± 1063	86

Day 18	18260± 2299	99	14855**± 1199	84

* significantly different from pre-dose values group (t test based on repeated measures analysis of variance) $p < 0.05$
** significantly different from placebo treated group $p < 0.05$.

Table 2	Individual RBC Cholinesterase Activity (IU) in volunteers Administered DDVP 0.1 mg/kg/day for 21 days								
	Volunteers								
	1[1]	2	3	4	5[1]	6	7	8	9[1]
Pre-dose Mean	18727	17204	17141	17480	17031	17069	16380	21156	19692
Day 1	17630 (94)	16940 (98)	17320 (101)	19380 (111)	16700 (98)	16180 (95)	15490 (95)	20460 (97)	19670 (100)
Day 2	18320 (98)	17150 (100)	16770 (98)	15550 (89)	16550 (97)	16430 (96)	15360 (94)	19640 (93)	19670 (100)
Day 4	19090 (102)	17280 (100)	16090 (94)	16650 (97)	16820 (99)	16370 (96)	15300 (94)	19910 (94)	20310 (103)
Day 7	18400 (98)	15230 (89)	15700 (92)	14570 (83)	16710 (98)	16790 (98)	15350 (94)	19910 (94)	20310 (103)
Day 9	17950 (96)	15080 (88)	16130 (94)	15180 (87)	17810 (105)	17640 (103)	14850 (91)	21370 (101)	19620 (100)
Day 11	19630 (105)	15680 (91)	15610 (91)	15020 (86)	18030 (106)	16300 (95)	14450 (88)	19160 (91)	19970 (101)
Day 14	19300 (103)	14500 (84)	15380 (90)	14580 (83)	16600 (97)	15300 (90)	14490 (88)	17750 (84)	19570 (99)
Day 16	18670 (100)	14710 (86)	15460 (90)	14530 (83)	14900 (87)	14420 (84)	14800 (90)	17230 (81)	19550 (99)
Day 18	19340 (103)	15800 (92)	15490 (90)	14680 (84)	15620 (92)	13310 (78)	13610 (83)	16240 (77)	19820 (101)
post-dose	17410 (93)	14130 (82)	13970 (82)	13840 (79)	16470 (97)	15190 (89)	13920 (85)	17310 (82)	19630 (100)

( ) indicates percent of individual predose mean, which was derived from 7 measurements done prior to dosing

[1] indicates placebo treated subject

DISCUSSION: As shown in Table 1, mean RBC cholinesterase activity was significantly reduced in DDVP treated male subjects when compared to control subjects on days 7, 11, 14, 16, and 18. However, when compared to the pre-treatment means, the mean RBC cholinesterase activity was reduced 14 percent on days 14 and 16, and 16 percent post dose on day 18. It should be noted that the pretreatment mean for the DDVP treated subjects was lower that of the placebo treated subjects. It should also be noted that even in the controls, there were up to 21 percent differences in individual RBC cholinesterase activity. The reduction in RBC cholinesterase activity was considered by the HAZARD ID Committee to be biologically significant. With respect to the use of male subjects only in this study, the NTP used lower doses in males in the mouse oncogenicity study based on information that males are more sensitive to the effects of DDVP than are females.

012541

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Secondary Reviewer: William Sette, Ph.D.
Science Analysis Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute/ Human volunteers

TOX. CHEM. No.: 328

MRID No.: 442488-02

GUIDELINE #: Non-guideline

TEST MATERIAL: 2,2,dichlorovinyl dimethyl phosphate

SYNONYMS: Dichlorvos, DDVP, Dedevap, Vapona

STUDY NUMBERS: XH 6064

SPONSOR: Amvac Chemical Corporation

TESTING FACILITY: Central Toxicology Laboratory, Adderly Park, Macclesfield, Cheshire, U.K.

TITLE OF REPORT: Dichlorvos: A study to investigate the effect of a single oral dose on erythrocyte cholinesterase inhibition in healthy male volunteers.

AUTHORS: Miss A.J. Gledhill

REPORT ISSUED: 25 March, 1997

EXECUTIVE SUMMARY: Dichlorvos was administered in a single oral dose of 70 mg (equivalent to 1 mg/kg) to fasted young healthy male volunteers. Prior to dosing, baseline RBC cholinesterase activity was measured on study days -22, -20, -18, -15, -13, -11, -8, -6, -4, and immediately prior to dosing. The study subjects were medically supervised for clinical signs and body temperature changes for twenty four hours and for RBC cholinesterase inhibition for up to fourteen days post compound administration.

Under the study conditions, no adverse clinical signs and no body temperature variations were reported. Mean RBC cholinesterase activity was statistically significantly inhibited, but the percent decrement was 12 percent or less on days 5/6, day 7, and day 14. No reduction in RBC cholinesterase activity was apparent

at other reporting periods. The reduction in RBC cholinesterase is considered to be biologically meaningful. **Under the study conditions, the LOEL for this study is 70 mg (equivalent to 1 mg/kg). This is a non-guideline study.**

MATERIALS:

Dichlorvos (lot number 608002S074) 988 pure was the test chemical. Corn oil Y007/007/007 was the vehicle. Red size 0 gelatin capsules were supplied by Zeneca Pharmaceuticals. Six healthy male volunteers age 21-30 years, weighing 67-80 kg were the test subjects.

METHODS:

1. Dosing preparation

Dichlorvos was dissolved at a concentration of 140 mg/mL in corn oil, so that the dose was contained in 500 L of corn oil, the capacity of the # 0 capsule. In order to ensure that the correct compound was prepared at the required concentration two staff persons were always present during the preparation of the dose formulation and the dosing capsules. The dose formulations were first passed through a sterile filter into a sterile container, then placed in sterile # 0 capsules which had previously been examined for Salmonella, Listeria, and general aerobic organisms such as Streptococcus and Staphylococcus. Capsules were stored individually in sterile containers. The dichlorvos content of the dosing formulations (both before and after capsule preparation) were analyzed by gas chromatography using a Hewlett Packard HP589A gas chromatograph with an electron capture detector and calibrated against a standard curve.

2. Study Design

Prior to study start, and at study termination, each participant was given a thorough medical history review and physical examination. Blood was withdrawn for standard hematology and biochemistry, determination of hepatitis B antigen, and human immunodeficiency virus. Heart rate, blood pressure, and EKG measured. Urine was collected for standard urinalysis and a of abuse screen. In addition, a drugs of abuse screen was conducted just prior to study start. During the three weeks p to dosing RBC cholinesterase activity was measured ten times (days -22, -20, -18, -15, -13, -11,-8, -6, -4, 0) for each individual in order to determine baseline values. On the day of dosing each volunteer was pre-fasted then administered gelatin capsules containing 70 mg of dichlorvos corn oil as a single oral dose. A blood sample was withdrawn cholinesterase determination immediately before dosing. The compound was administered with 150 mL distilled water

with volunteers sitting upright. Volunteers remained overnight in testing facility for 24 hours post compound administration ul. constant medical supervision. Body temperature was recorded pre-dose and at 2, 4, 8, 12, and 24 hours after dosing. Any clinical signs observed were reported on adverse events forms by the study investigator. Blood was withdrawn for determination of cholinesterase activity at 24, 48, 72, 120/144, 168, and 336 hours post dosing.

For cholinesterase activity testing, 10 mL of blood was withdrawn into heparinized tubes, and centrifuged at 2000 rpm at ambient temperature for 15 minutes to separate plasma and red blood cells. The red blood cells were transported in insulated containers to CTL immediately after the plasma had been removed. All cholinesterase determinations were done immediately upon the receipt of the samples. Plasma was snap frozen using dry ice and acetone and stored in polypropylene screw top vials at -201 C.

Clinical procedures were performed at Medeval Limited, University of Manchester, U.K. Laboratory determinations were done at Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, U.K.

3. Criteria for Study Inclusion

To be included in the study volunteers had to meet the following criteria: be between the ages of 18 and 45 and of a single ethnic group; have normal clinical examination including history and EKG; clinical chemistry, hematology, and urinalysis within the normal range; RBC cholinesterase activity above 10000 IU/L: negative screen for serum hepatitis B and HIV; negative screen for drugs of abuse; weigh between 60 and 90 kg, and not vary by more than 15% from the desirable weight as described by Metropolitan Life Tables.

4. Restrictions on Study Participants

Study participants were required to do the followings avoid exposure to organophosphate compounds during and for two months after the study; abstain from taking medication from 24 hours before or until 48 hours after dosing; abstain from driving or operating machinery on the dosing day; abstain from smoking, caffeine containing drinks before each dosing day and until 24 hours after dosing; abstain from alcohol for 24 hours before and for 5 days after dosing; abstain from strenuous physical activity, including sports for 72 hours before dosing until 72 hours after dosing; abstain from potential hazardous activity for 48 hours after dosing; fast from 12 midnight on the night before dosing; remain in the testing unit for 24 hours after dosing. Participants were free to withdraw from the study at any time.

PROTECTION OF SUBJECTS: The study was conducted in accordance with the Declaration of Helsinki including all amendments up to and including the Hong Kong revision. The study protocol was approved by the Medeval Independent Ethics Committee, University of Manchester, U.K.

QUALITY ASSURANCE: Signed and dated quality assurance statements were included with the submission.

STATISTICAL ANALYSIS: Group means for each time point were compared using a paired t-test(SAS, 1989)

Differences between pre-study and subsequent cholinesterase levels for each individual at each time point were compared using the Goode permutation test.

RESULTS: Analysis of the dosage formulations as well as the test capsules demonstrated that the dose achieved was within two percent of target concentration. No clinical signs were observed and no symptoms were reported to the medical staff during the overnight period of residency, or to the study investigator at the medical examination prior to release from the unit 24 hours after administration of DDVP. Body temperatures were not affected by administration of DDVP.

When the group mean RBC cholinesterase activity was analyzed, there were statistically significant reductions from the predose mean on days 5/6, day 7, and day 14. These represented percent changes of 10, 12, and 11 percent, respectively. The individual predose values used to calculate the mean RBC cholinesterase activity varied by 17 percent for volunteer 1, 16 percent for volunteer 2, 6 percent for volunteer 3, 10 percent for volunteer 4, 7 percent for volunteer 5, and 9 percent for volunteer 6. The group mean statistical analysis is shown below in Table 1

Table 1 Mean RBC cholinesterase activity (I.U) in Male Volunteers administered 70 mg DDVP in a Single Oral dose

	Pre-dose	Day 1	Day 3	Day 5/6	Day 7	Day 14
Group Mean	18559± 2531.31	17457± 2100	17893± 2432	16773** ±2154	16387** ±1771	16537** +2268
% predose group mean	100	94	96	90	88	89

Over the fourteen day observation period, the post test RBC cholinesterase activity value for each individual ranged from 82 to 101 percent of the mean pretest value (ten pretest values from each volunteer) as shown in Table 2, taken from the investigators' report.

Table 2. RBC Cholinesterase Activity (I.U.) in Human Volunteers Administered A Single Oral Dose of Dichlorvos 70 mg

Time	Subjects					
	I	II	III	IV	V	VI
Pre-Test (Mean)	23214	18754	19084	17393	16584	16361
Day 1	21020 (91%)	16280 (87%)	18550 (97%)	16740 (96%)	17180 (101%)	14970 (91%)
Day 3	22170 (96%)	17400 (93%)	18550 (97%)	17570 (101%)	16840 (102%)	14830 (91%)
Day 5/6	20910 (90%)	16120 (86%)	17300 (91%)	15320 (88%)	15350 (93%)	15460 (94%)
Day 7	19830 (85%)	15940 (85%)	16620 (87%)	15440 (89%)	15540 (93%)	15050 (92%)
Day 14	20800 (90%)	15440 (82%)	17390 (91%)	14940 (86%)	15580 (94%)	15070 (92%)

( ) indicates percent of individual RBC ChE activity as compared to the individual's mean predose ChE's value.

DISCUSSION: The data presented demonstrate that under the study conditions, administration of a single oral dose of dichlorvos did not produce any change in body temperature, or any reported clinical symptoms. Group mean RBC cholinesterase activity was statistically significantly altered on days 5/6 7, and 14. These values, however, were within 10, 12, and 11 percent of pretest values respectively, but were considered biologically meaningful by the HAZARD ID SARC.

Under the study conditions, LOEL for RBC cholinesterase inhibition is 70 mg, equivalent to 1 mg/kg. A NOEL has not been determined.