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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: May 27, 2020

SUBJECT: Summary data evaluation record (DER) for three OCSPP non-guideline studies; *In Vitro* Inhibition Kinetics of 17 Organophosphates on Human and Rat Erythrocyte Acetylcholinesterase.

PC Code: See table below
Decision No.: 459247
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0058039
MRID No.: 50773501, 50773502, 50773503

DP Barcode: D451226
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: See table below
40 CFR: NA

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THROUGH: Monique Perron, Sc.D., Toxicologist
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Health Effects Division (7509P)

TO: Lauren Bailey, Chemical Review Manager
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Pesticide Reevaluation Division (7508P)

CHEMICAL	PC CODE	CAS NUMBER
Bensulide	009801	741-58-2
Chlorethoxyfos	129006	54593-83-8
DDVP	084001	62-73-7
Dicrotophos	035201	141-66-2
Dimethoate	035001	60-51-5
Ethoprop	041101	13194-48-4
Fenamiphos	100601	2224-92-6
Malathion	057701	121-75-5
Methamidophos	101201	10265-92-6
Naled	034401	300-76-5
Omethoate	035002	1113-02-6
Parathion	057501	56-38-2
Phorate	057201	298-02-2
Phosmet	059201	732-11-6
Phostebupirim	129086	96182-53-5
Terbufos	105001	13071-79-9
Tribufos	074801	78-48-8

I. ACTION REQUESTED:

Please review and prepare data evaluation record (DERs) for three non-guideline studies submitted evaluating inhibition kinetics of organophosphates (OPs) on rat and human erythrocyte acetylcholinesterase (MRIDs 50773501, 50773502, 50773503).

II. BACKGROUND:

Three non-guideline, *in vitro* inhibition kinetic studies have been submitted to the Agency (MRIDs 50773501, 50773502, 50773503). The objective of the studies was to measure the kinetic constants (bimolecular rate constant k_i, dissociation constant K_I, and phosphorylation constant k_p) describing the inhibition of acetylcholinesterase by 17 OP pesticides or OP oxons. The comparison of the kinetic parameters generated between humans and rats is intended to address the interspecies uncertainty in risk assessment by estimating interspecies pharmacodynamic data-derived extrapolation factors (DDEFs). Similarly, the range of values obtained in these experiments from individual humans is intended to address the intraspecies uncertainty of these OP compounds by estimating intraspecies pharmacodynamic DDEFs. Furthermore, the kinetic constants derived for malaoxon and omethoate were generated for use in physiologically-based pharmacokinetic (PBPK) modeling.

III. RESULTS:

A summary DER was composed for the three non-guideline, *in vitro* inhibition kinetic studies submitted to evaluate inhibition kinetics of 17 OPs on rat and human erythrocyte acetylcholinesterase (MRIDs 50773501, 50773502, 50773503), which includes an overview of the methods and presents the range of human and rat k_i values for each OP compound, except tribufos, which did not produce any AChE inhibition even at a final concentration of 1 mM.

EPA Reviewer: Julian Pittman, Ph.D. **Signature:**
Risk Assessment Branch VI, HED (7509P) **Date:** 4/06/2020

EPA Secondary Reviewer: Monique Perron, Sc.D. **Signature:**
Risk Assessment Branch I, HED (7509P) **Date**: 5/12/2020

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DATA EVALUATION RECORD

STUDY TYPE: OCSPP Non-guideline; *In Vitro* Inhibition Kinetics of 17 Organophosphates on Human and Rat Erythrocyte Acetylcholinesterase.

PC CODES: 009801, 034401, 035001, 035002, 035201, 041101, 057201, 057501, 057701, 059201, 074801, 084001, 100601, 101201, 105001, 129006, 129086

DP BARCODE: D451226

TXR#: 0058039

TEST MATERIAL (PURITY): 83.6% to 99.8% (depending on chemical)

CITATION: Chambers, J., Meek, E., Crow, J.A. (2018) Inhibition Kinetics of Malaoxon and Omethoate on Human and Rat Erythrocyte Acetylcholinesterase. December 20, 2018. MRID 50773501. Unpublished.

SPONSOR: FMC Corporation 2929 Walnut St. Philadelphia, PA 19104

CITATION: Chambers, J., Meek, E., Crow, J.A. (2018) Inhibition Kinetics of 13 Organophosphates on Human and Rat Erythrocyte Acetylcholinesterase. December 20, 2018. MRID 50773502. Unpublished.

SPONSOR: AMVAC Chemical Corporation 4695 MacArthur Court, Suite 1200, Newport Beach, CA 92660

CITATION: Chambers, J., Meek, E., Crow, J.A. (2018) Inhibition Kinetics of Bensulide Oxon and Phosmet Oxon on Human and Rat Erythrocyte Acetylcholinesterase. December 20, 2018. MRID 50773503. Unpublished.

SPONSOR: Gowan Company, LLC PO Box 5569 Yuma, AZ 85366

EXECUTIVE SUMMARY:

Three non-guideline, *in vitro* inhibition kinetic studies (MRID 50773501, 50773502, 50773503) were submitted for review. The objective of the studies was to measure the kinetic constants (bimolecular rate constant k_i, dissociation constant K_I, and phosphorylation constant k_p) describing the inhibition of acetylcholinesterase (AChE) by 17 organophosphate (OP) pesticides or OP oxons:

- MRID 50773501: malaoxon (the active metabolite of malathion) and omethoate (the active metabolite of dimethoate)
- MRID 50773502: DDVP, naled, dicrotophos, tribufos, phorate oxon sulfone (phorate metabolite), phorate oxon sulfoxide (phorate metabolite), ethoprop, methamidophos, fenamiphos, terbufos oxon sulfone (terbufos metabolite), terbufos oxon sulfoxide (terbufos metabolite), chlorethoxyfos oxon (chlorethoxyfos metabolite), and tebupirimphos oxon (tebupirimphos metabolite)
- MRID 50773503: bensulide oxon (the active metabolite of bensulide) and phosmet oxon (the active metabolite of phosmet).

Additionally, paraoxon was tested as a positive control. The comparison of the kinetic parameters generated between humans and rats are intended to address the interspecies uncertainty in risk assessment by estimating interspecies data-derived extrapolation factors (DDEFs). Similarly, the range of values obtained in these experiments from individual humans are intended to address the intraspecies uncertainty in the kinetics of these OP compounds by estimating intraspecies DDEFs. Furthermore, the kinetic constants derived for malaoxon and omethoate were generated for use in physiologically-based pharmacokinetic (PBPK) modeling.

The assays were performed using "erythrocyte ghost" preparations (i.e., erythrocyte cell membranes separated from hemoglobin and other cytoplasmic constituents) that were obtained from either human or rat erythrocytes as the source of AChE. The most important measurement was identified as k_i calculated via the hyperbolic method. Rat and human k_i values are reported for each OP compound below, except tribufos, which did not produce any AChE inhibition even at a final concentration of 1 mM.

METHODS:

Test substances and positive control (paraoxon) were obtained from multiple sources with purity ranging from 83.6% to 99.8%.

The kinetic constants were determined for 18 individual human samples (9 adults, 5 juveniles, and 4 cord blood samples). Human AChE utilized in the experiments was derived from blood samples from individual healthy humans of both sexes (adults age 16-60, and juveniles age 10-13), as well as cord blood samples. Blood samples from multiple race and ethnic groups were included in the study reports.[1]

The kinetic constants were also determined for three individual pooled samples from adult rats (Sprague Dawley [Crl:CD(SD)BR]. Each of three male samples was prepared from the pooled blood of five male rats. Similarly, each of three female samples was prepared from the pooled blood of five female rats. The positive control for optimization of the kinetics assay procedures and comparative purposes was paraoxon. The control substance was initially characterized as a single band by preparative thin layer chromatography, and it displayed consistent levels of AChE inhibition throughout the study.

[1] MRID 50773501 pp. 11-13; MRID 50773502 pp. 15-17; MRID 50773503 pp. 11-12.

A continuous spectrophotometric assay was used to determine AChE activities (modification of Ellman et al., 1961) with acetylthiocholine (ATCh) as the substrate and 5,5'-dithiobis (nitrobenzoic acid) (DTNB) as the chromogen (Chambers et al., 1988). The assays used a BioTek M5 microplate reader with Gen 3 Software (BioTek Instruments, Winooski, Vermont). The software measured the absorbance in each well and calculated the velocity of each reaction by determining the slope of the line from a plot of absorbance (proportional to product formed) as a function of time.

Following the incubation with vehicle or OP, the inhibition reaction was terminated by addition of the substrate ATCh. The AChE activity remaining following the inhibition by OP was determined by the addition of ATCh and DTNB. The reaction was linear over the time assayed with no inhibitor present, indicating no significant depletion of substrate. The reaction was also linear at the highest concentration of inhibitor for the entire reaction time, indicating no ongoing inhibition following the addition of substrate.

For each species and each inhibitor, all regressions and calculations were performed using either Microsoft Excel 2010 or SigmaPlot version 14 to obtain the AChE velocity remaining ($[E]_t/[E]_o$) and apparent rate of AChE phosphorylation (k_{app}) for determination of k_i, k_p, and K_I.

RESULTS:

The range of human and rat k_i values for each OP compound, except tribufos, which did not produce any AChE inhibition even at a final concentration of 1 mM, are presented below in Table 1.The larger the k_i, the greater the capacity of an OP compound to inhibit AChE.

Table 1.

OP	Human k_i ($L \cdot mol^{-1} \cdot min^{-1}$)		Rat k_i ($L \cdot mol^{-1} \cdot min^{-1}$)	
	Low	High	Low	High
DDVP	3.50×10^4	6.94×10^4	3.70×10^4	6.58×10^4
Naled	9.30×10^5	8.23×10^6	3.38×10^6	1.33×10^7
Dicrotophos	3.88×10^3	7.37×10^3	3.23×10^3	1.11×10^4
Phorate oxon sulfone	1.18×10^4	1.23×10^5	6.04×10^4	1.50×10^5
Phorate oxon sulfoxide	3.16×10^4	7.03×10^4	4.66×10^4	9.51×10^4
Ethoprop	8.88×10^2	2.44×10^3	4.83×10^2	1.11×10^3
Methamidophos	5.82×10^2	1.95×10^3	5.37×10^2	3.47×10^3
Fenamiphos	7.00×10^1	2.19×10^2	7.37×10^1	3.07×10^2

OP	Human k_i ($L \cdot mol^{-1} \cdot min^{-1}$)		Rat k_i ($L \cdot mol^{-1} \cdot min^{-1}$)	
	Low	High	Low	High
Terbufos oxon sulfone	2.49×10^5	1.05×10^6	2.12×10^5	4.06×10^5
Terbufos oxon sulfoxide	7.48×10^3	2.94×10^4	6.69×10^3	9.96×10^3
Chlorethoxyfos oxon	1.36×10^7	3.66×10^7	4.42×10^7	7.84×10^7
Tebupirimphos oxon	2.11×10^5	8.34×10^5	7.10×10^5	2.48×10^6
Malaoxon	0.078×10^5	2.196×10^5	0.419×10^5	1.173×10^5
Omethoate	0.279×10^3	5.831×10^3	1.328×10^3	3.730×10^3
Bensulide oxon	0.2070×10^3	0.635×10^3	0.231×10^3	0.932×10^3
Phosmet oxon	0.434×10^5	2.053×10^5	0.560×10^5	1.421×10^5
Paraoxon (positive control) MRID 50773501, 50773502, 50773502	6.714×10^5	10.24×10^5	N/A	N/A 8.47×10^5 (Carr and Chambers (1996))

DEFICIENCIES:

MRID 50773501, 50773502, 50773503:

Regarding the whole blood samples from human donors, there was an unusual spread of age, ethnicity and gender. Furthermore, comparisons within age, ethnicity and gender may be limited by small sample sizes. Also, because pooled rat samples were used from one rat strain, it is difficult to determine the variability within the rat species, either among individuals or strains.

MRID 50773502:

There are numerous bimolecular rate constants (k_i) that have significant outliers associated with them (across OPs (human/rat data)), as compared to the other two study reports.