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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: 16-DEC-2016

SUBJECT: **Carbaryl.** Review and generation of Data Evaluation Record

PC Code: 056801
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0057550
MRID No.: NA

DP Barcode: D437064
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 63-25-2
40 CFR: NA

FROM: Sarah S. Gallagher, Ph.D., Biologist
Risk Assessment Branch I (RABI)
Health Effects Division (HED) (7509P)

THROUGH: Chris Olinger, Acting Branch Chief
Risk Assessment Branch I (RABI)
Health Effects Division (HED) (7509P)

Monique Perron (for)

TO: Linsey Walsh, Chemical Review Manager
Pesticide Re-evaluation Division

I. CONCLUSIONS

RABI has reviewed the human study for carbaryl and classified it as an acceptable/non-guideline study.

II. ACTION REQUESTED

Please review the attached document – May, et al. (1992). "Cimetidine-Carbaryl Interaction in Humans: Evidence of an Active Metabolite of Carbaryl."

EPA Reviewer: Sarah S. Gallagher, Ph.D. **Signature:**
RAB1, Health Effects Division (7509P) **Date:** 12/16/16
EPA Secondary Reviewer: Monique M. Perron, Sc.D. **Signature:**
RAB1, Health Effects Division (7509P) **Date:** 12/16/16

Template version 03/12

TXR#: 0057550

DATA EVALUATION RECORD

STUDY TYPE: Special Study: Cholinesterase Activity Resulting from Carbaryl and Cimetidine Exposure.

PC CODE: 056801 **DP BARCODE:** D437064

TEST MATERIAL (PURITY): Carbaryl

SYNONYMS: α-naphthyl-N-methyl carbamate

CITATION: May, D.G. (1992) Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl. *J Pharmacol Exp Ther*. **262** (3) 1057-1061.

EXECUTIVE SUMMARY:

In this *in vivo* study, four non-smoking, drug-free normal males were dosed with carbaryl and, at another time, carbaryl following pre-treatment with cimetidine. Carbaryl concentrations were measured in plasma at 0, 10, 20, 30, 45, 60, 90, 120, 150, 210, and 240 minutes following exposure, and the area under the plasma concentration-time curve was estimated. Oral clearance was calculated. Red blood cell (RBC) acetylcholinesterase (AChE) activity was determined at each time point. This study did not measure any metabolites of carbaryl.

The results of the *in vitro* assays confirmed that, at a concentration of 10 or 100 µg/mL, cimetidine did not impact the RBC AChE inhibition caused by increasing concentrations of carbaryl.

Following oral administration of 1 mg/kg carbaryl, the mean peak plasma concentration was 0.26 ± 0.14 µg/ml at 30 minutes after exposure. The mean plasma half-life for carbaryl was 0.79 ± 0.47 hours. The apparent oral clearance for carbaryl (5.4 ± 2.0 L/min) was greater than would be expected for liver blood flow. The maximal reduction in RBC AChE activity (27 ± 12%) was measured at 30 minutes after dosing. The RBC AChE activity recovery half-life was approximately 2.6 ± 1.5 hours, which was slower than the plasma half-life.

One week after the first dose of carbaryl, the same four male subjects were treated with 200 mg of cimetidine every 8 hours for 3 days. On the third day, 1 mg/kg of carbaryl was administered one hour after the last dose of cimetidine. Pre-treatment with cimetidine reduced the clearance for carbaryl by 50% which resulted in a 2-fold increase in peak plasma concentration. These findings support the hypothesis that the main site of metabolism for carbaryl is the liver by drug-metabolizing enzymes that can be inhibited by cimetidine. Despite the increased systemic availability, the concentration of carbaryl required to achieve a 20% inhibition of AChE activity

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increased by over an order of magnitude in the presence of cimetidine.

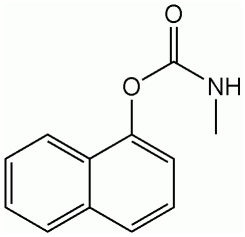
This study is **Acceptable/Non-Guideline**.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Carbaryl

Description: Not Reported.
Lot/batch #: Not Reported.
Purity: Not Reported.
Compound stability: Not Reported.
CAS # of TGAI: 63-25-2
Structure:



2. Vehicle and/or positive control: Four mL of polyethylene glycol (PEG) and 100 mL of water.

3. Test animals:

Species: Human/Males
Strain: NA
Age/weight at study initiation: 24-43 years old. Body weights were not reported.
Source: Not Reported.

B. STUDY DESIGN:

1. In life dates: Not Reported.

2. Subject assignment: All subjects received same dosing regimen (Table 1).

TABLE 1: Dosing groups for pharmacokinetic studies for SAN 1315H. [a]

Exp. #	Test group	Carbaryl Nominal Dose (mg/kg)	Dose Route	Remarks
1	Carbaryl only treatment	1.0	Oral	Plasma samples were collected at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after dosing with carbaryl.
2	Carbaryl treatment following pre-treatment with cimetidine	1.0	Oral	Subjects were pre-treated with 200 mg of cimetidine every 8 hr for 3 days. On the third day, carbaryl was given 1 hr after the last dose of cimetidine. Plasma samples were collected at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min after dosing with carbaryl.

[a] Data obtained from page 1058 in the journal article.

3. Dose selection rationale: The doses were based on results of previous studies that found that normal human subjects are able to tolerate carbaryl at doses of 0.1, 0.5, and 1.0 mg/kg without symptoms[1].

[1] Knaak et al. (1968). Metabolism of carbaryl in man, monkey, pig, and sheep. J. Agric. Food Chem. 16: 465-470.

C. METHODS:

1. ***In Vitro* Assays:** *In vitro* assays were conducted to measure the effect of carbaryl, cimetidine, and a mixture of carbaryl and cimetidine on RBC AChE activity. All incubations were run in duplicate with the coefficient of variation less than 10% between duplicates. For the first set of assays, a solution of carbaryl in PEG was added to a buffered suspension of human RBCs to final concentrations ranging from 0 to 10 µg/ml and incubated for one minute. Then, the RBC AChE activity was measured using the Ellman technique, a colorimetric assay for AChE activity. For the second set of assays, cimetidine was added to a buffered suspension of RBCs to final concentrations ranging from 7.5 to 300 µg/ml. After a one-minute incubation, RBC AChE activity was measured using the Ellman technique. For the third set of assays, an aqueous solution of cimetidine was added to a final concentration of 10 or 100 µg/ml. After a 10-minute incubation, carbaryl was added to final concentrations ranging from 0 to 10 µg/ml and incubated for one minute. After the incubation, RBC AChE activity was measured.

   Calculations: The percent of inhibition was calculated using the following equation:

$$\%\ Inhibition = \frac{Activity_{BLANK} - Activity_{CONC.}}{Activity_{BLANK}} \times 100$$

2. ***In Vivo* Exposure:** Each subject was dosed on two separate occasions with a single oral administration of carbaryl. The first treatment was only carbaryl. The second treatment of carbaryl was administered following a 3-day pre-treatment period with cimetidine. Cimetidine is an H_2-histamine receptor blocker that is known to reduce the activity of cytochrome P450 isozymes. Pre-treatment with cimetidine was conducted to allow for the evaluation of the contribution of cytochrome P450s to carbaryl metabolism.

   After each dose of carbaryl, blood was collected at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes. HPLC was used to measure the plasma carbaryl, and the area under the plasma concentration-time curve was estimated using the log trapezoidal method. Oral clearance was estimated by dividing the dose by the area under the curve with extrapolation to infinity. Extrapolation was accomplished using least squares regression analysis of the terminal log concentration time curve to define the terminal exponential. RBC AChE activity was also measured at each time point using the Ellman technique. The results are presented as the percentage inhibition as compared to the control, which was the RBC AChE activity level in the subjects prior to administration of carbaryl.

3. **Statistics:** Mean values were reported with standard deviations. Statistical comparisons of results from the two arms of the study utilized analysis of variance, repeated measures design, with $P < 0.05$ accepted as significant. An effort was made to obtain the original study data; however, the data were not available so an analysis of the statistics was not able to be performed.

II. RESULTS:

A. *IN VITRO* STUDIES:

1. **Influence of Carbaryl and Cimetidine on RBC AChE Activity:** Incubation of carbaryl with RBCs produced a dose-dependent inhibition of AChE activity, with 1 µg/mL resulting in a 20% inhibition (Figure 1). Cimetidine was also shown to induce a dose-dependent inhibition of RBC AChE activity; however, a 50-fold greater concentration of cimetidine was required to reach 20% inhibition (Figure 1). In the absence of carbaryl, cimetidine concentrations of 10 µg/mL (equivalent to plasma levels observed in humans at therapeutic doses) and 100 µg/mL resulted in a 15% and 50% inhibition of RBC AChE activity, respectively. For 10 µg/mL of cimetidine, increasing concentrations of carbaryl, ranging from 0.1 to 10 µg/mL, resulted in RBC AChE inhibition that was similar to the assays using just carbaryl (Figure 2).

FIGURE 1. Inhibition of RBC AChE activity in isolated human erythrocytes by carbaryl (circles) and cimetidine (squares).

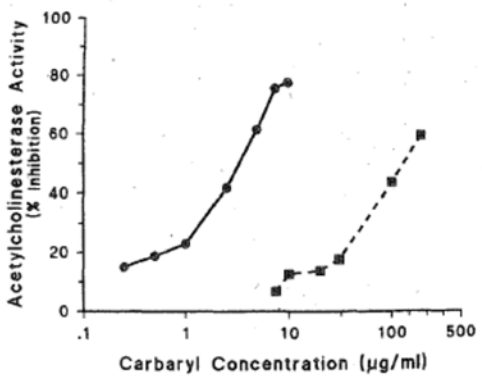
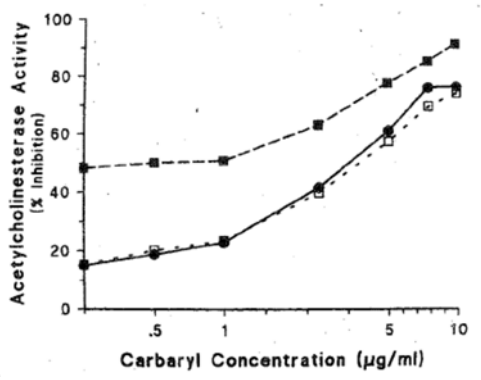


FIGURE 2. Inhibition of RBC AChE activity in isolated human erythrocytes by carbaryl (black circles), carbaryl with 10 mg/mL cimetidine (white squares) carbaryl with 100 mg/mL cimetidine (black squares).



B. *IN VIVO* STUIDES:

1. **Influence of Cimetidine on Carbaryl Disposition:** Following oral administration of 1 mg/kg carbaryl, the mean peak plasma concentration was 0.26 ± 0.14 µg/ml at 30 minutes after exposure (Table 2; Figure 3). The plasma half-life for carbaryl was 0.79 ± 0.47 hours.

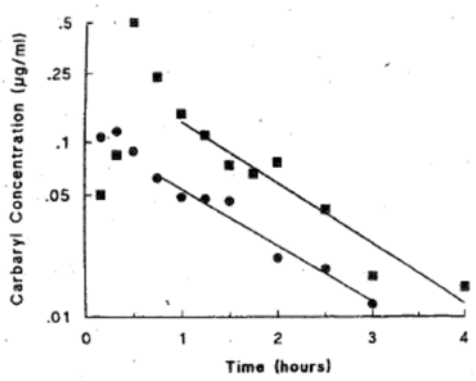
The apparent oral clearance for carbaryl (5.4 ± 2.0 L/min) was greater than would be expected for liver blood flow. The maximal reduction in RBC AChE activity (27%) was measured at 30 minutes after dosing (Table 2; Figure 4). The RBC AChE activity recovery half-life was approximately 2.6 ± 1.5hr, which was slower than the plasma half-life.

TABLE 2. Carbaryl pharmacokinetics and pharmacodynamics and the effect of cimetidine.

	Carbaryl	Carbaryl and Cimetidine
Pharmacokinetics		
Peak plasma concentration (µg/mL)	0.26 ± 0.14	0.55 ± 0.26
Oral clearance (L/min)	5.4 ± 2.0	2.5 ± 1.5*
Plasma half-life (hr)	0.79 ± 0.47	1.24 ± 0.75
Pharmacodynamics		
Maximal change in acetylcholinesterase activity (% inhibition)	27 ± 12	14 ± 6*
Half-life of inhibition (hr)	2.6 ± 1.5	1.3 ± 1.3

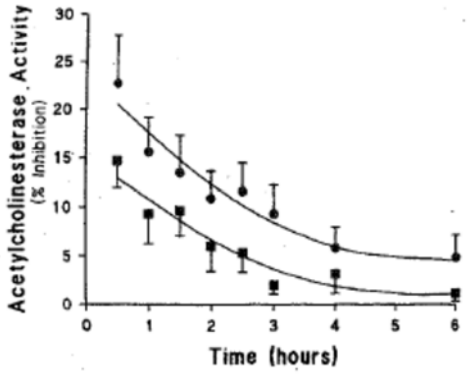
* $P < 0.05$

FIGURE 3. The plasma concentration of carbaryl following oral administration of carbaryl (1 mg/kg) alone (circles) and following pretreatment with cimetidine (squares).



2. **<u>Influence of Cimetidine on Carbaryl Dynamics</u>:** Pre-treatment with cimetidine for 3 days prior to carbaryl administration resulted in a 2-fold increase in peak plasma concentrations, a 50% reduction in apparent oral clearance, and relatively no change in the plasma half-life (Table 2). Despite the increased systemic availability, the maximal reduction in RBC AChE activity caused by 1 mg/kg carbaryl was not as great following cimetidine pre-treatment (14 ± 6%; Figure 4). The RBC AChE activity recovery half-life was also shorter (1.3 ± 1.3hr) than treatment when carbaryl was given alone.

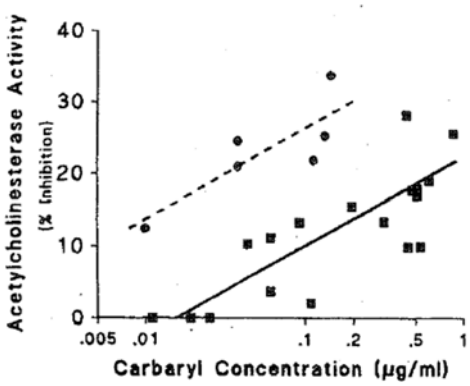
FIGURE 4. RBC acetylcholinesterase activity following oral administration of carbaryl (1 mg/kg) alone (circles) and following pretreatment with cimetidine (squares).



3. **<u>Relationship Between Plasma Carbaryl Concentration and AChE Activity</u>:** Oral administration of varying concentrations of carbaryl established a clear relationship between carbaryl concentration in plasma and RBC AChE activity, with a concentration of approximately 0.02 µg/ml inducing a 20% reduction in RBC AChE activity (Figure 4). In the presence of cimetidine, there was still a clear dose-response relationship; however, there was a parallel shift of the plasma concentration-response curve to the right, and a 1 µg/mL was required to produce a 20% inhibition.

   By comparing the *in vivo* results to those obtained from the *in vitro* assays, it was shown that a 50-fold greater concentration of carbaryl was required to induce a 20% reduction in AChE activity *in vitro*, than is required to produce a similar response *in vivo*. While a plasma concentration in humans of 0.02 µg/mL was required to induce a 20% reduction, equivalent inhibition in isolated RBC could be achieved with approximately 1 µg/mL (Figure 1).

   FIGURE 4. The relationship between plasma concentration of carbaryl and RBC acetylcholinesterase activity following oral administration of carbaryl (1 mg/kg) alone (circles) and following pretreatment with cimetidine (squares).



III. DISCUSSION:

This study was conducted to measure the pharmacokinetic and pharmacodynamic response of RBC AChE to 1 mg/kg of carbaryl alone as well as the effect of administration of 1 mg/kg of carbaryl following pre-treatment with cimetidine. RBC AChE inhibition was also measured *in*

vitro in isolated RBCs. Both *in vitro* and *in vivo*, carbaryl induced a concentration-dependent reduction in RBC AChE activity.

The results of the *in vitro* assays confirmed that in the presence of 10 or 100 µg/mL of cimetidine, RBC AChE inhibition caused by increasing concentrations of carbaryl was similar to inhibition caused by carbaryl alone. These findings support the conclusion that there is not a synergistic interaction between carbaryl and cimetidine.

Following oral administration of 1 mg/kg carbaryl, the mean peak plasma concentration was 0.26 ± 0.14 µg/ml at 30 minutes after exposure. The mean plasma half-life for carbaryl was 0.79 ± 0.47 hours. The apparent oral clearance for carbaryl (5.4 ± 2.0 L/min) was greater than would be expected for liver blood flow. The maximal reduction in RBC AChE activity (27 ± 12%) was measured at 30 minutes after dosing. The RBC AChE activity recovery half-life was approximately 2.6 ± 1.5 hours, which was slower than the plasma half-life.

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