UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Washington, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 4/14/06

SUBJECT: **Human Studies Review Board**: **CARBOFURAN** Weight-of-the-Evidence Presentation of Human and Animal Toxicity Studies

PC Code: 090601

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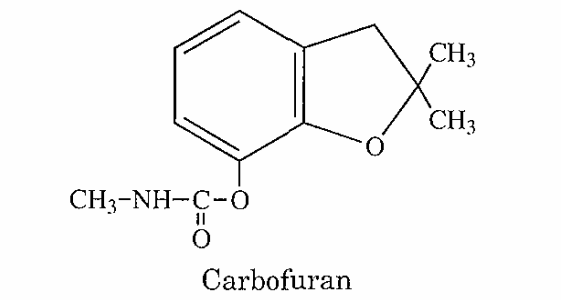
The purpose of this weight-of-evidence (WOE) report is to present the study design, methods, and results of three different human studies to the Human Studies Review Board (HSRB) to evaluate them individually and collectively for their scientific validity. The human studies that will be presented are a single oral dose study and two single dose dermal studies. Relevant animal data are also summarized and conclusions as to how the Office of Pesticide Programs (OPP) Health Effects Division (HED) proposes to use these data in both the single chemical assessment and the *N*-methyl carbamate cumulative assessments are presented.

Introduction

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) is a broad spectrum *N*-methyl carbamate (see structure below) is an insecticide that can cause cholinergic toxicity through inhibition of the acetylcholinesterase(s) of the peripheral and/or central nervous system. Similar to other *N*-methyl carbamate pesticides, inhibition is followed by rapid recovery of acetylcholinesterase. Animal toxicity studies (dog, rat, mouse) using both acute and chronic exposure conditions show that the primary toxicologic effect following carbofuran exposure by the oral route is cholinesterase (ChE) activity inhibition, the most sensitive endpoint. Due to the rapid recovery of the inhibition, results of chronic studies in the dog, rat, and mouse indicate that the effects of carbofuran do not accumulate over time

Several toxicity studies involving direct dosing of humans with carbofuran (including both the oral and dermal routes of exposure) have been conducted. ChE inhibition and associated clinical signs have been demonstrated in the submitted human volunteer studies.



Carbofuran

TOXICITY STUDIES

HUMAN

1) Human Single-Dose Oral Study:

Arnold, J.D. (1976*) Evaluation of the Safe Exposure Levels to Carbamate, Administered Orally to Healthy Adult Normal Male Volunteers.* (Unpublished study received Oct 24, 1979 under 279- 2712; prepared by Quincy Research Center, submitted by FMC Corp., Philadelphia, Pa.; CDL:241303-B) Accession no. 241303. MRID 00092826.

Study Description

Laboratory: Quincy Research Center, Kansas City, MO

Study Dates: Not reported; study report is dated 9/17/1976

Purpose of Study: "To determine the threshold toxicity level in normal male volunteers to single oral doses of carbamate."

General design: The protocol for this study called for an ascending dose schedule with the following doses: 0.05, 0.1, 0.25, 0.50, 1.0, and 2.0 mg/kg. Part A was an "open" experiment (assumed to mean that both investigator and subject were aware that carbofuran was being ingested) that started with two subjects each receiving 0.05 mg/kg carbofuran with no placebo group. If no toxicity was observed after 24 hours, two more subjects were then given the next dose, and this was repeated until "intolerance was demonstrated". Then Part B of the experiment was performed in which one subject received a placebo and two subjects received the highest (i.e., intolerable) dose observed in Part A. In Part B, the design was both randomized and double blind. Table 1 shows the study design up to the dose considered intolerable.

Subjects were admitted to the Quincy Research Ctr. by 4 pm the evening before the study began, and were dosed the following morning *after* a standard breakfast. According to the protocol, the placebo and carbofuran capsules were provided by the Midwest Research Institute [MRI], but how they were consumed by the subjects is not recorded. The volunteers were confined to their beds from

0 hour to 2 hrs post-dosing, and were permitted only minor ambulatory activity from 2 hours to 8 hours post-dosing. The subjects remained under observation for 24 hours, and were permitted no alcohol or concomitant medications during the course of the study. When the observation period ended, the pre-entry physical examinations and laboratory determinations were repeated and the subjects released from the center. Cigarette smoking was permitted during the study (2 of the 4 high-dose subjects were the only non-smokers).

Subjects: Nine healthy men "who fulfilled the selection criteria were selected to participate in the study." No information was provided about how or from what pool they were recruited. The age and body weight ranges were 23-47 and 60-95 kg, respectively.

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Table 1: Single Oral Dose Study Design

Study Phase (Session)		Dose (mg/kg)			
		Control	0.05	0.1	0.25
Part A	(1)	None	2	–	–
	(2)		–	2	–
	(3)		–	–	2
Part B		1	–	–	2

Measures: RBC and Plasma cholinesterase activity (0–hour/pre-dosing, and then 30 min., 1, 2, 3, 6 , and 24 hrs post-dosing); EKG, blood pressure, heart rate, temperature, respiration, vestibular function, pupil size, eye accommodation, hematology, urinalysis and blood chemistry were measured at pre-dosing and at various intervals up to 24 hours after dosing. During the observation period (24 hours) subjects were monitored for signs or symptoms of toxicity. The next highest dosing was not initiated until data from the 24-hour post treatment were evaluated.

The cholinesterase assay used in this study (Voss and Sachsse, 1970) was a modification of the Ellman colorimetric method. This method uses propionylthiocholine rather than acetylthiocholine as the substrate in the assay. According to the abstract (see Appendix 1), this method was developed to assess cholinesterase activities in humans when a small volume of blood is collected for analysis.

Analysis: There was no statistical analysis of the data.

Study Results

Table 2 summarizes the results of the study. The lowest dose tested (0.05 mg/kg) resulted in minimal RBC ChEI with no symptoms being reported. The second and third doses, however, showed a marked decrease in RBC ChEI and in symptoms (both subjective and objective) in a dose-related manner. The third dose (0.25 mg/kg) was considered an "intolerable" dose in the first two subjects tested and so was tested in another two subjects as Part B of the experiment, along with a single control subject. Appendix 2 provides the individual RBC ChEI values (in terms of percent change from baseline) for each subject at each time point (from pre-dose to 24 hours). The data show both the decrease in RBC at the 1-3 hour post-dosing time period and the eventual return to "normal" values. Plasma ChEI values are not presented in Appendix 2 as they were more variable and the RBC ChEI is considered more important as a marker for carbofuran exposure.

It should be noted that EPA performed a benchmark (BMD) analysis of this human data as part of the *N*-methyl cumulative assessment and calculated a $BMDL_{10}$ of 0.026 mg/kg (BMD_{10} of 0.039 mg/kg).

Table 2: Results of Single Oral Human Study with Carbofuran

Dose	RBC ChEI[1]	Plasma ChEI	Symptoms
Control	10%	54%	No symptoms noted.
0.05 mg/kg	11%, 22%[2]	32%, 36%	
0.1 mg/kg	33%, 31%[2]	56%, 35%	Headache (1 subject); lightheadedness (the other subject). One subject had EKG anomalies (25% decrease in sinus rate, sinus arrhythmia, and sinus bradycardia – all at 4 hr and 24 hr post-treatment time points). Same subject showed deterioration in vestibular mechanism (but baseline was already abnormal and the subject was a smoker).
0.25 mg/kg	46%, 63%, 62%, 59%[3]	33%, 100%, 53%, 81%	Symptoms usually attributed to cholinesterase inhibition (drowsiness, nausea, dizziness, nervousness, vomiting, headache, weakness, dry mouth, salivation, unsteadiness[4], abdominal pains, and diaphoresis) were seen in 3 of 4 subjects within 3 hrs of dosing. The fourth exhibited nausea within a 30 min. of dosing. Three of the subjects had changes in heart rate, lower body temperature, and lower respiration rates. EKG results showed anomalies in three subjects (sinus bradycardia, 25% decrease in sinus rate) – one subject did not recover by the end of the monitoring period (24 hrs).

[1] Percent decrease provided for each individual subject on study. Maximum inhibition presented for all subjects.
[2] Seen at 1-hr; almost returned to normal by 3 hr in all subjects.
[3] Seen at 1 [one subject] and 3 [three subjects] hrs post dosing; almost returned to normal by six hrs.
[4] Vestibular mechanisms for 2/4 were abnormal and deteriorating one hr. post-dosing. One of these subjects could not stand up after one hour post-dosing.

Strengths/Uncertainties/Limitations

The following strengths of the single dose oral study in male human volunteers have been identified:

- Effects were observed in the treated subjects (in terms of both subjective and objective measures).
- Multiple doses were used.

- All subjects were monitored (they were kept at the center from the night before dosing up to 24 hours after dosing).
- The ChEI effects observed (and associated symptoms) occurred at or about the expected peak time (1-3 hours post-dosing) and returned to almost normal within six hours of dosing.

The following uncertainties/limitations have also been identified:

- There was no discussion of the rationale for dose selection.
- The number of subjects is low (2 subjects) for the low- (0.05 mg/kg) and mid-dose (0.1 mg/kg) groups, and a placebo (with only one subject) was only used in Part B of the experiment.
- All tested subjects were males.
- There was no statistical analysis.
- Part A was "open" – assumed to mean that both the investigator and subject were aware that the subject(s) were receiving the treatment capsule.
- There was potential confounding due to smoking (all except two subjects – both in the 0.25 mg/kg group – smoked). See Appendix 2 for number of cigarettes consumed by each subject during the study.
- Duplicate blood samples were taken to analyze for carbofuran and/or its metabolites but the data have not been submitted.
- The RBC/plasma ChEI method used was a different method than what is normally used. The study report is dated 1976 and it is assumed that the study was performed around that time. The method paper was published in 1970 (Voss, G. and Sachsse, 1970). The Ellman method was published in 1961.
- No justification or rationale of dose selection

Conclusions

The study authors considered the dose level of 0.1 mg/kg as a "NOEL" whereas the OPP has considered the dose level of 0.1 mg/kg as a "LOEL" in the past[1].

There is some confidence in the high dose (0.25 mg/kg) as a definitive effect level. Although there may be potential confounding due to smoking, this dose level had 4 subjects that demonstrated clear symptoms of cholinesterase toxicity, and moreover, the symptoms could be correlated in their severity and incidence with the extent and duration of RBC ChEI. Effects consisted of drowsiness, nausea, dizziness, nervousness, vomiting, headaches, weakness, dry mouth, salivation, unsteadiness, abdominal pains, and diaphoresis. Changes in heart rate, lower body temperatures, and lower respiration rate were also noted in these subjects. There were also effects on EKG, vestibular mechanisms, pupillary constriction and eye accommodation.

At the 0.1 mg/kg dose level, one subject had a headache, and the other was occasionally lightheaded. The study authors did not consider the headache and lightheadedness to be compound related. One subject exhibited a decrease in sinus rate. This subject also exhibited deterioration in the vestibular mechanism; although the baseline value for this parameter was abnormal to begin with. In addition, the one concurrent control in the second round of testing at 0.25 mg/kg displayed decreased sinus rate and erratic vestibular function (at baseline it was borderline between normal and abnormal, and at various time intervals was reported as abnormal and deteriorating). Therefore, the sinus rate and vestibular function effects are difficult to assess.

Using the BMD calculation, the $BMDL_{10}$ of 0.026 mg/kg is a reasonable Point of Departure (PoD) for possible use in risk assessment.

[1] In 1997, OPP requested an external peer review of this carbofuran study. Three academic scientists reviewed the study and determined it was an appropriate study to use to develop an RfD and also commented on the use of uncertainty factors in deriving an RfD (Burnam, 1970).

2) Human Dermal Study:

Arnold, J.D. (1977) *Carbamate (Carbofuran) Human Dermal Study*. Final rept. (Unpublished study received Oct 24, 1979 under 279-2712; prepared by Quincy Research Center, submitted by FMC Corp., Philadelphia, Pa.; CDL:241303-C), Accession no. 241303. MRID 00092827.

Study Description

Laboratory: Quincy Research Center, Kansas City, MO

Study Dates: Not reported; study report is dated 1977.

Purpose of Study: "To determine the threshold toxicity level to simple and multiple doses of Carbofuran under normal and elevated temperatures and/or humidities"

General design: Like the single oral dose study described above, this dermal study was designed as an ascending dose study. The protocol proposed to begin with a dose of 0.5 mg/kg, with dosing increasing in multiples of two up to 16 mg/kg (note that the study went up to 32 mg/kg) until the "minimum effect level" is reached. The minimum effect level is defined as "…the level which produces mild but definitive clinical symptoms, as observed in the oral studies." After a standard breakfast, volunteers were dermally dosed with a 50% test article dilution (undiluted test substance: Furadan 75 WP; 75% carbofuran) on a designated portion of their upper backs[2], mildly exercised for 5 minutes, then allowed to rest for 15 minutes. This cycle was repeated 11 times over 4 hours. After four hours, the carbofuran was removed/washed from the back. With each cycle, the dose was doubled until a "minimum effect level" was attained. After the 4 hour dosing interval, the dosages were removed and subjects monitored for the remainder of the 24 hour post-treatment period. Cigarette smoking was permitted during the study

Subjects: Eighteen healthy males (19-53 years of age) were chosen with a weight range of 63-102 kg.

Numbers/Doses: This study was performed in three parts—A, B, and C. Eight subjects were assigned to part A, eight subjects were assigned to part B, and two subjects were assigned to part C (see Table 3 below). Escalating doses were used in parts A (0, 0.5, 1.0, and 2.0 mg ai/kg) and B (2.0, 4.0, 8.0, and 32.0 mg ai/kg) over the course of one day (2 subjects per dose level; *single* dermal dose) using high temperature/high humidity (85-95 degrees F and 68-89% relative humidity) in Part A and normal temperature/normal humidity (70-75 degrees F and 35-40% relative humidity) in Part B. Part C consisted of treating two test subjects once daily at 1.0 mg ai/kg/day on three consecutive days *(multiple* dermal doses) under the same high temperature/high humidity conditions identified above for Part A.

Table 3: Acute Dermal Study Design (Arnold, 1977)

Study Phase (Session)	Dose (mg/kg)						
	Control	0.5	1.0	2.0	4.0	8.0	32.0

[2] The submitted report is unreadable regarding specifics on this procedure (see page 113 in the electronic copy of Accsn241303, also labeled as page 129 on the bottom right hand corner of the page).

A[1]	2	2	2	2	-	-	-
B[2]	-	–	-	2	2	2	2
C[1]	-	–	2	-	-	-	-

[1] High temperature/high humidity conditions.

[2] Normal temperature/normal humidity conditions.

Measures: RBC and Plasma cholinesterase activity (0–hour/pre-dosing,1, 2, 3, 6 , and 24 hours); EKG, blood pressure, heart rate, temperature, respiration, vestibular function, pupil size, eye accommodation, hematology and urinalysis were measured at pre-dosing and at various intervals up to 24 hours after dosing. During the observation period (24 hours) subjects were monitored for clinical signs. As noted above for the oral study, the Voss and Sachsse method for ChEI determination was used.

Analysis: There was no statistical analysis of the data.

Study Results

Tables 4-6 show the results from Parts A, B, and C; respectively. RBC Cholinesterase inhibition was compared to the self pre-dose measurement. The subjects given the high dose of 2 mg/kg in Part A were the only ones from any of the three experiments that showed both appreciable decreases in RBC ChEI and symptoms generally associated with a cholinergic response. Because this exposure scenario was under high heat/high humidity conditions, it is likely that this contributed to the enhanced absorption and associated toxicity. This can be inferred given that the results of the same experiment performed under normal heat/humidity conditions (Part B) using much higher doses (up to 32 mg/kg) showed no effects. The three-day dosing experiment summarized in Table 3 was inconclusive since there were no effects and no controls. In all three experiments, male volunteers were required to perform mild exercise activities which could be seen as enhancing the possible dermal absorption and possible toxicity of carbofuran.

Table 4: Results of Dermal Human Study (Arnold, 1977) with Carbofuran: Part A[1]			
Dose	**RBC ChEI[2]**	**Plasma ChEI[2]**	**Symptoms**
Control	10%, 10%	0, 25%	No symptoms noted.
0.5 mg/kg	20%, 23% (both @ 3 hrs.)	10%, 10% (@6 and 3 hrs)	
1.0 mg/kg	39%, 9% (@ 3 and 1 hrs)	5%, 11% (both @ 3 hrs)	
2.0 mg/kg	46%, 65% (both @ 4 hrs)	12%, 16% (both @ 24 hrs)	One subject (46% peak RBC ChEI) reported feeling weak and shaky 3 hrs, 30 min. post-dosing and was administered atropine at 5 hrs post-dose. The other subject had more severe symptoms (lightheadedness, hazy vision, hunger, vomiting, defecation with muscular cramps, chills) from 2 hrs, 30 min. to 4 hrs post dosing. Atropine was administered at three different times (4 hrs 20 min, 5 hrs, 5 min; and 5 hrs, 15 min. post-dosing. The carbofuran solution was washed off his back prior to atropine administration. Both subjects had changes in pulse and vestibular function. EKGs were normal and pupil size/accommodation measurements could not be made. Both subjects were asymptomatic at 24 hrs post-dosing.

[1] Single dose of carbofuran applied to backs of volunteers. Subjects were placed in an enclosed environment with high temperature/high humidity conditions one hour before dosing and then for four hours post-dosing. During the four hour exposure period, they exercised for 5 minutes, rested for 15 minutes and then continued this cycle 11 times.

[2] Percent decrease provided for each individual. Maximum inhibition presented for all individuals and the time point associated with that maximum.

Table 5: Results of Dermal Human Study (Arnold, 1977) with Carbofuran: Part B[1]			
Dose	**RBC ChEI[2]**	**Plasma ChEI[2]**	**Symptoms**
2.0 mg/kg	10%, 20% (both @ 6 hrs.)	6%, 5% (@ 6 and 3 hrs)	No symptoms noted.
4.0 mg/kg	13%, 11% (@ 6 and 2 hrs.)	26%, 33% (both @ 2 hrs)	
8.0 mg/kg	7%, 17% (@ 3 and 6 hrs)	+7%, 4% (@ 3 and 1/6 hrs)	
32.0 mg/kg	24%, 16% (@ 8 and 4 hrs)	0, 2% (both @ 10 hrs)	

[1] Single dose of carbofuran applied to backs of volunteers. Subjects were placed in an enclosed environment with normal temperature/normal humidity conditions one hour before dosing and then for four hours post-dosing. They then exercised for 5 minutes, rested for 15 minutes and then continued this cycle 11 times (four hours).
[2] Percent decrease provided for each individual. Maximum inhibition presented for all individuals and the time point associated with that maximum.

Table 6: Results of Dermal Human Study (Arnold, 1977) with Carbofuran: Part C[1]			
Dose	**RBC ChEI[2]**	**Plasma ChEI[2]**	**Symptoms**
1.0 mg/kg	26%, 29% (both @ 24 hrs. on day 2)	43%, 49% (both @ 4 hrs on day 1)	No symptoms noted.

[1] Single dose of carbofuran applied to backs of volunteers for three successive days. Subjects were placed in an enclosed environment with high temperature/high humidity conditions one hour before dosing and then for four hours post-dosing. They then exercise for 5 minutes, rest for 15 minutes and then continued this cycle 11 times (four hours).
[2] Percent decrease provided for each individual. Maximum inhibition presented for all individuals and the time point associated with that maximum.

Strengths/Uncertainties/Limitations:

The following strengths of the Arnold, 1977 dermal study in male human volunteers have been identified:

- Effects were observed in the treated subjects in Part A of the study (in terms of both subjective and objective measures).

- Multiple doses were used.
- All subjects were monitored (they were kept at the center from the night before dosing up to 24 hours after dosing).
- The ChEI effects observed occurred at or about the expected peak time for dermal exposure (3-4 hours post-dosing; a lag associated with dermal exposure vs. oral) and returned to almost normal within six hours of dosing.

The following uncertainties/limitations have also been identified:

- The number of subjects is low (2 subjects) and placebos were only used in one of the three experiments (Part A).
- All tested subjects were males.
- There was no statistical analysis.
- All three experiments appear to have been "open" – assumed to mean that both the investigator and subject were aware that the subject(s) were receiving either the placebo or the treatment capsule.
- There was potential confounding due to smoking because it was permitted, but unlike the oral study described above, no details were provided on which subject smoked or how much they may have smoked.
- The RBC/plasma ChEI method used was a different method than what is normally used (as mentioned above as an uncertainty in the oral study).
- Duplicate blood samples were taken to analyze for carbofuran and/or its metabolites but the data have not been submitted.
- Individual data missing from the study report included clinical signs, pupil size and eye accommodation, pulse rate, blood pressure, EKG, respiration rate, body temperature, vestibular function, and clinical pathology.
- No rationale or justification for doses selected

Conclusions

Although effects were observed in Part A of this study, it is important to note that the subjects were under atypical conditions (high temperature and high humidity) and were asked to perform mild exercise (stationary bike) for five minutes eleven times over a four hour period while the carbofuran was on their backs. The effects observed at the highest dose tested (2 mg/kg) resulted in both marked RBC ChEI and cholinergic symptoms that required intervention with the antidote atropine in both of the subjects tested at this dose. Similar symptoms were not observed under normal temperature/humidity conditions – at much higher doses (up to 32 mg/kg) but following the same exercise regimen. However, it is believed that the study has value in that it attempts to understand dermal absorption and possible toxicity to carbofuran under high heat/humidity – conditions that may exist in certain occupational settings (e.g., outdoor agricultural working conditions). Thus, OPP/HED believes these data may be useful in a hazard assessment as a PoD (with a LOAEL of 0.5 mg/kg given the RBC ChEI observed in Part A – see Table 4).

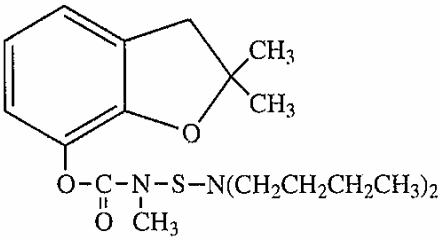
2b) Arnold, J.D. (1978) *Comparison of Cholinesterase Inhibition and Effects of Furadan 4F and FMC 35001 4 EC* (ACT 152.03). Rev. final rept. (Unpublished study received Oct 24, 1979 under 279- 2712; prepared by Quincy Research Center, submitted by FMC Corp., Philadelphia, Pa.; CDL:241305-A) Accession no. 241305, MRID no. 00092829.

Laboratory: Quincy Research Center, Kansas City, MO

Study Dates: Not reported; study report is dated 1978.

Purpose of Study: "The objective of this study was to compare the effects of single cutaneous applications of Furadan 4F (4F) and FMC 35001 4EC (EC) in men exposed to high temperature and humidity. Specific goals were: [to determine]….(1) effects of treatment on plasma and erthyrocyte cholinesterase activity, (2) ….the minimal dose level …which induces symptoms of cholinesterase inhibition, (3)….the effects of the two treatments on neurovegetative signs…".

(NOTE: Furadan 4F is carbofuran and FMC 35001 4EC is carbosulfan, a carbofuran analog – see structure below).



Carbosulfan

General design: In an open, parallel study design, volunteers were dermally dosed with a 50% test article dilution [undiluted test substance: carbofuran or carbosulfan] on a designated portion of their upper backs (0.5 mg per square centimeter) approximately 1 ½ hours after a standard breakfast. Subjects received Furadan 4F or FMC 35001 4EC in groups of two; no subject received more than one treatment of either chemical. From 0-4 hours post-treatment, subjects mildly exercised for 5 minutes, then were allowed to rest for 15 minutes. The exercise/rest cycle was repeated 11 times over 4 hours. Subjects were kept in environmentally controlled rooms of high temperature and high humidity (91-96 degrees F and relative humidity 70-80%). After the 4 hour dosing interval, the dosages were removed (soap and water) and subjects monitored for the remainder of the 24 hour post-treatment period. Cigarette smoking was not allowed 1/2 hour before treatment and until 2 ¼ hours after dose administration.

Subjects: Twenty males (eight received carbofuran and 12 received carbosulfan) were used with an age range of 18-53 years old; and had a weight range of 1?? – 188 pounds [lower value unreadable in submitted data].

Numbers/Doses: Groups of 2 subjects were dosed with carbofuran at 0.5, 1.0, 2.0, or 4.0 mg/kg. Groups of 2 subjects were dosed with carbosulfan at 0.5, 1.0, 2.0, 4.0, 8.0, or 16.0 mg/kg.

Table 7: Acute Dermal Study Design: Arnold, 1978[1]							
Study Phase (Session)	Dose (mg/kg)						
	Control	0.5	1.0	2.0	4.0	8.0	16.0
Carbofuran	-	2	2	2	2	-	-
Carbosulfan	-	2	2	2	2	2	2

[1] All subjects were placed in a room under high temperature/high humidity conditions for the four hour exposure period.

Measures: RBC and Plasma cholinesterase activity (0–hour/pre-dosing, ½, 1, 2, 4, 6 , and 24 hours); EKG, blood pressure, heart rate, temperature, respiration, pupil size, eye accommodation, hematology and urinalysis were measured at pre-dosing and at various intervals up to 24 hours after dosing. During the observation period (24 hours) subjects were monitored for clinical signs. As noted above for the oral study, the Voss and Sachsse method for ChEI determination was used.

Analysis: There was no statistical analysis of the data.

Study Results

For the purposes of this WOE, only the carbofuran data are presented in Table 8. RBC ChE inhibition values were based on self-pretest levels. The data show clear cholinergic effects (decrease in RBC ChEI and associated symptoms) at the high dose of 4 mg/kg. As with the dermal study described above, both treated subjects at this dose received an antidote due to the severity of the response. Although the 4 mg/kg reported here represents a two-fold difference in carbofuran amount as compared to the 2 mg/kg reported for the other dermal study (Arnold, 1977), the differences are likely due to the fact that Arnold (1978) uses a formulation (includes a higher concentration of inert ingredients) with 4% carbofuran and the previously reported dermal study uses a product that contains 75% carbofuran.

Table 8: Results of Carbofuran Data From Comparative Dermal Study[1]			
Dose	**RBC ChEI[2]**	**Plasma ChEI[2]**	**Symptoms**
0.5 mg/kg	22%, 7%	33%, 46% (@1 and 4 hrs)	One subject reported nausea [at 40 min and 2 hrs 10 min. post-dosing]. The first episode lasted 10 min and the second about 40 min. The other subject reported a burning sensation at application site that lasted about 5 min [at 1 hr, 50 min. post-dosing].
1.0 mg/kg	29%, 21%	4%, 15% (@ 6 and 1 hrs)	No symptoms reported
2.0 mg/kg	42%, 40%	44%, 6% (@ 2 and 24 hrs)	
4.0 mg/kg	61%, 49%	6%, 9% (@ 30 min and 1 hr)	Both subjects reported nausea, dizziness and weakness. One also reported "swollen tongue", increased salivation, and stomach cramps; and the other experienced vomiting and tremors. Most symptoms began about 3 hrs. post-dosing and disappeared within 2 hrs of onset. In both cases atropine was administered as an antidote [in the first case, at 4 hours and in the second at 3 hrs]. In addition, the second subject's back was washed to remove the treatment at 3 hrs [1 hour early] and the subject was removed from the high temp/high humid room.

[1] Single dose of carbofuran applied to backs of volunteers. Volunteers are placed in an enclosed environment under high temperature/high humidity conditions. They then exercise for 5 minutes, rest for 15 minutes and then continue this cycle 11 times (four hours).
[2] Percent decrease provided for each individual. Maximum inhibition presented for all individuals. For RBC values, the maximum inhibition was seen at the 4-hr time point in all cases.

Strengths/Uncertainties/Limitations:

The following strengths of the Arnold, 1978 dermal study in male human volunteers have been identified:

- Effects were observed in the treated subjects (in terms of both subjective and objective measures).
- Multiple doses were used.
- All subjects were monitored (they were kept at the center from the night before dosing up to 24 hours after dosing).
- The ChEI effects observed occurred at or about the expected peak time (4 hours post-dosing; a lag associated with dermal exposure vs. oral and in agreement with the values measured in Arnold, 1977) and returned to almost normal within six hours of dosing.

The following uncertainties/limitations have also been identified:

- Only two volunteers were treated at each dose level.

- The study design was open (so both investigators and subjects knew that all volunteers were receiving carbofuran).
- There were no control subjects.
- There were no statistical analyses.
- Only males were tested.
- All subjects were placed in a high temperature/high humidity environment and were required to exercise
- The carbofuran product used was a formulation (i.e., 4% carbofuran) and so contains other ingredients at high concentrations that confound the results of this study (especially when compared with the Arnold (1977) study described earlier which used a 75% technical produce).

Conclusions

The comparative dermal toxicity study showed apparent cholinergic effects at 4 mg/kg, but not at 2 mg/kg. However, comparison of RBC ChE inhibition data from the two dermal studies show similar degrees of inhibition at the same doses (0.5, 1.0, and 2.0 mg/kg). The dermal study described earlier showed effects at 2 mg/kg (the highest dose tested in that study). The study designs were essentially similar between the two studies, with the major difference between the two being the use of a technical product (75% carbofuran in the first study) and the use of a formulation (4% carbofuran) in this study may explain the different results since the carbofuran formulation that contains numerous inert or other ingredients that could inhibit dermal absorption.

Together, the two dermal studies suggest a dermal LOAEL of 0.5 mg/kg based on RBC ChEI at the expected peak time (3-4 hours) with the appropriate recovery over the next six hours. Thus, this dose may be used as a PoD for an appropriate occupational dermal exposure scenario.

ANIMAL

Consistent with the other *N*-methyl carbamates, the effects from carbofuran exposure do not accumulate over time. This lack of accumulation is due to the rapid recovery of the acetylcholinesterase. Therefore, the most appropriate exposure route is via gavage while dietary studies are less appropriate.

There are several studies available where acute ChE inhibition was measured at or near peak time of inhibition (15-45 min) and which inform the derivation of the acute RfD in the rat. These include two studies performed by the registrant: 1) time course study (MRID no. 45675701) where male and female rats were dosed at 0.5 and 1.0 mg/kg and 2) recently submitted comparative ChE study where adult and juvenile (post-natal day 11, PND) rats were dosed at 0.3, 0.6, and 1.0 mg/kg (MRID no. 46688914). Clinical signs were also reported for adults and PND11 pups in the comparative ChE study. In support of the Agency's cumulative risk assessment for the *N*-methyl carbamates, scientists from the National Health and Environmental Effects Research Laboratory (NHEERL) performed dose-response studies in male rat where brain and RBC ChE inhibition along with motor activity were measured. For carbofuran, the Agency's study included doses ranging from 0.1 mg/kg up to 1.5 mg/kg (USEPA, 2005).

Table 9. Benchmark estimates for carbofuran from the comparative ChE study and the Preliminary Cumulative Risk Assessment for the N-methyl carbamate pesticides.

Source	Brain		RBC	
	Male	Female	Male	Female

Adult (CCA)	**BMD_{10}**	0.11	0.12	Data provides no dose-response relationship	
	$BMDL_{10}$	0.05	0.06		
Preliminary Cumulative RA	**BMD_{10}**	0.15	N/A	0.03	N/A
	$BMDL_{10}$	0.13		0.01	
Human-Oral	**BMD_{10}**	NA	NA	0.038	NA
	$BMDL_{10}$	NA	NA	0.025	NA

PROPOSED USE IN SINGLE CHEMICAL ASSESSMENT

OPP HED proposes that the single oral dose human study ($BMDL_{10}$ of 0.026) be used directly as a point of departure. Based on RBC ChE inhibition at the lowest dose tested (0.5 mg/kg) in both dermal studies, and using a LOAEL to NOAEL extrapolation factor of 3X, the point of departure is 0.17 mg/kg for worker exposure.

PROPOSED USE IN *N*-METHYL CARBAMATE CUMULATIVE ASSESSMENT

1. Background:

The Food Quality Protection Act (FQPA) was passed by Congress in 1996. The FQPA made key changes to the approaches used by EPA to assess pesticide chemicals. One of these changes was the requirement to consider cumulative risk to those pesticides which act by a common mechanism of toxicity. Pesticides are determined to have a "common mechanism of toxicity" if they act the same way in the body--that is, the same toxic effect occurs in the same organ or tissue by essentially the same sequence of major biochemical events. OPP established the *N*-methyl carbamate pesticides (NMCs) as a common mechanism group and in accordance with FQPA has developed a preliminary cumulative risk assessment for this group of pesticides (USEPA, 2005). Carbofuran is a member of the NMC common mechanism group.

OPP has developed a guidance document for developing cumulative risk assessments under FQPA (USEPA, 2002). This guidance indicates that when developing a multi-chemical hazard assessment, comparison of toxic potency should be made using a uniform basis of comparison, by using to the extent possible a common response derived from a comparable measurement methodology, species, and sex for all the exposure routes of interest. In the preliminary cumulative risk assessment, the Agency considered RBC and brain ChE inhibition as potential endpoints. Plasma cholinesterase data were not considered since the primary enzyme in plasma is butylcholinesterase and not acetylcholinesterase. Ultimately, brain ChE data from acute rat toxicity studies measured at or near the time of peak effect have been used by EPA to estimate a relative potency factor (RPF) and to develop the points of departure (PoD) for extrapolating cumulative risk. For instance, the brain BMD_{10} has been used to calculate the RPF while the brain $BMDL_{10}$ establishes the PoD in the preliminary cumulative risk assessment. Brain data have been selected over RBC data as brain ChE inhibition represents a direct measure of the target tissue (as opposed to blood data which is considered a surrogate measure) and brain ChE inhibition data tend to have less variation and thus confer less uncertainty on cumulative risk estimates.

Because data from rat studies provide the basis for potency determination, the Agency must consider interspecies extrapolation (i.e., animal to human) in its cumulative risk assessment. As such, human data may be used by the Agency to inform the pesticide-specific interspecies extrapolation. In the specific case of carbofuran, MRID 00092826 is available.

2. Carbofuran Human Study Summary:

The single oral human toxicity study for carbofuran does not provide brain ChE data, for obvious reasons, but does provide RBC ChE data for male volunteers. The blood ChE activity (RBC) provided in the human study is considered appropriate surrogate measures of potential effects on peripheral nervous system (PNS) acetylcholinesterase (AChE) activity, and of potential effects on the central nervous system (CNS) when brain ChE data are lacking (USEPA 2000). AChE is the target enzyme for the cumulative risk assessment and is the primary form of ChE found in RBCs. Butylcholinesterase (BChE), on the other hand, is the primary for of ChE found in plasma. BChE is considered a measure of exposure but has not been shown to be of toxicological significance. As with the RBC ChE data from the aldicarb, methomyl, and oxamyl human studies that were presented to the HSRB in April 2006, the RBC ChE data from the single oral carbofuran human study also is being utilized by the Agency to inform the pesticide-specific interspecies extrapolation.

The measured RBC ChE activity from the oral human study is adequate for estimation of BMD and BMDL estimates. The RBC ChE data from the carbofuran human study was utilized in the model in the same manner as the RBC data from the oral human studies for aldicarb, methomyl, and oxamyl. The BMD_{10} and $BMDL_{10}$ estimates for both rat (RBC, brain) and human (RBC) are included in Table 9 . It should be noted that the BMD estimates for the rat include both sexes while the BMD estimates from the human study only include male subjects.

Based on the FIFRA SAP (2005) approval of statistical analyses for the BMD model, the single RBC BMD estimate for carbofuran indicates similar ChE activity in males and females (0.031 mg/kg, M and F). Comparison of the rat BMD estimates from the two compartments indicates the RBC compartment is slightly more sensitive than the brain. The RBC ChE data from the human study, therefore, would also be expected to be protective of brain ChE inhibition. As such, the BMD estimate from the carbofuran human study provides useful information into the sensitivity of RBC ChE inhibition of rats compared to humans.

3. Discussion

Several studies for carbofuran are available that provide quality dose-response and ChE inhibition data from both the rat and human. As presented for aldicarb, methomyl, and oxamyl at the April 2006 HSRB, the cumulative risk assessment for carbofuran also must extrapolate information across compartments (blood-brain). The preliminary NMC cumulative risk assessment relies on rat brain ChE data for the relative potency factors (RPFs) and points of departure (PoDs). The FIFRA SAP supported the Agency's use of brain ChE data in August 2005 (FIFRA SAP 2005).

Similar to the approach proposed by the Agency at the April, 2006 for aldicarb, methomyl, and oxamyl for informing the interspecies extrapolation factor in the cumulative risk assessment, the carbofuran human study may also inform the interspecies extrapolation factor for the preliminary cumulative risk assessment. The ratio of the rat BMD_{10} to the human BMD_{10} was proposed at the April 2006 HSRB. The Agency is proposing to use the same approach for carbofuran. The Agency is in the process of analyzing both the rat and human BMD_{10} data to determine the central estimate and 95% confidence interval for use as the interspecies extrapolation factor. A rough estimate of the interspecies extrapolation factor for carbofuran may be made by comparing the RBC BMD_{10} values for the rat and human from Table X above. This ratio is approximately 1X. The interspecies extrapolation factor resulting from the formal BMD ratio analysis would be in addition to the other uncertainty factors for the cumulative risk assessment that includes intraspecies variability (human variability) and FQPA.

References

Voss, G and K Sachsse. 1970. *Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine/DTNB procedure* Toxicology and Applied Pharmacology, 16:764-777.

Burnam, W. 1997. "Discussion of Outside Peer Review Comments on the Carbofuran RfD". Memorandum to Tina Levine entitled "

FIFRA SAP (2005). Meeting Minutes of the FIFRA Scientific Advisory Panel Held August 23-26, 2005. SAP Minutes No. 2005-04. Preliminary N-Methyl Carbamate Cumulative Risk Assessment. October 13, 2005.

USEPA (2000). "The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides"; August 18, 2000. Available: http://www.epa.gov/pesticides/trac/science/cholin.pdf

USEPA (2002). "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity." January 14, 2002. (67 FR 2210; January 16, 2002) http://www.epa.gov/oppfead1/trac/science/#common

USEPA (2005). Preliminary N-Methyl Carbamate Cumulative Risk Assessment. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, DC. http://www.epa.gov/scipoly/sap/index.htm#sept

APPENDIX 1

Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine/DTNB procedure

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Abstract

A modification of the acetylthiocholine/DTNB method has been developed for the simultaneous determination of red cell and plasma cholinesterases in samples of only 0.01–0.03 ml of whole human and animal blood, using a relatively short incubation period of 10 min and an incubation temperature of 30°C. In contrast to many other micromethods, these test conditions allow activity determinations of cholinesterases inhibited by insecticidal carbamates *in vivo*, because decarbamylation of the enzymes after dilution is comparatively low after 10 min at 30°C. Human blood cholinesterases are assayed with propionylthiocholine, whereas for blood samples of various laboratory animals acetylthiocholine was found to be the better substrate. The experimental procedure of this micromethod is based on two cholinesterase measurements, of which the first determines total activity in whole blood, and the second, plasma cholinesterase activity alone. Erythrocyte cholinesterase activity is determined by subtraction. Normal activity values of the enzymes in red cells, plasma, and brain are given for a variety of species, and a few *in vivo* inhibition data after organophosphate and carbamate poisoning are also presented.

APPENDIX 2

Appendix 2: RBC ChEI Data (Percent Change from Baseline) in Human Single Oral Dose Study									
Time of Blood Sample	**1[1]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	0.05 mg/kg		**0.1 mg/kg**		**0.25 mg/kg**				**Placebo**
Pre	-	-	-	-	-	-	-	-	-
30 min	-8	+5	-29	-15	-21	-5	+12	-??[2]	+6
1 hr	-22	-11	-33	-31	-58	-63	-46	-59	-10
2 hr	-	-	-	-	-46	-49	???[2]	-52	+3
3 hr	+5	+3	-4	-2	-62	-57	-12	-26	-1
6 hr	+8	+7	0	+12	-8	0	+6	+5	+28
24 hr	+12	+5	+1	+5	+24	+27	+51	+44	+40

[1] Subject number. Subjects 1 and 2 received 0.05 mg/kg, subjects 3 and 4 received 0.1 mg/kg, subjects 5 through 8 received 0.25 mg/kg and subject 9 was the lone placebo in the study.
[2] Value unreadable on data table in submitted report.

Appendix 2: Number of Cigarettes Consumed During the Human Single Oral Dose Study									
Time Post-Dosing	**1[1]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	0.05 mg/kg		**0.1 mg/kg**		**0.25 mg/kg**				**Placebo**
0-1 hr	0	0	0	0	0	0	0	0	0
1-2 hr	3	2	2	0	0	0	0	1	1
2-3 hr	2	2	0	2	0	0	1	1	1
3-4 hr	1	0	1	2	0	0	0	0	1
4-5 hr	1	2	1	1	0	0	0	1	2.5
5-6 hr	1	2	0	0	0	0	0	0	2
6-7 hr	0	2	2	0	0	0	1	1	1
7-8 hr	1	1	1	2	0	0	1	2	3
TOTAL	**9**	**11**	**7**	**7**	**0**	**0**	**3**	**6**	**11.5**

[1] Subject number. Subjects 1 and 2 received 0.05 mg/kg, subjects 3 and 4 received 0.1 mg/kg, subjects 5 through 8 received 0.25 mg/kg and subject 9 was the lone placebo in the study.