Bayer CropScience

September 10, 2004

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 South Bell Street
Arlington, Virginia 22202-4501

Attention: Mr. Anthony Britten, SRRD

Dear Mr. Britten,

Re: **Carbaryl Interim Reregistration Eligibility Decision (IRED)**
Risk Assessment in Support of Liquid Broadcast Applications to Residential Lawns/Turf

Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
Phone: 919 549-2000

Please find enclosed the final report of the post-application risk assessment conducted in support of the liquid broadcast application of carbaryl products to residential lawns. This report supersedes the draft assessment sent to you on June 15, 2004.

The conclusion developed from application of the carbaryl pharmacokinetic data in this refined risk analysis is that the revised lawn reentry MOEs for children are in excess of 100, i.e., there is reasonable certainty of no harm associated with the use of carbaryl-based broadcast lawn care products on residential turf when label directions are followed.

Three (3) copies of the following documents are enclosed:

Volume 1 of 2: Transmittal Document

Volume 2 of 2: Ross, J., J. Driver and C. Lunchick. (2004) Application of Carbaryl Pharmacokinetic Data in the Estimation of Potential Post-Application Health Risks Associated with Broadcast Lawn Care Products. Non-Guideline Document. infoscientific.com, Inc. Project Identification No. is.c 04 Bayer101. Document No. B004716. September 8, 2004. 40pp.

Please let me know if you need any additional information. My phone number is (919) 549-2718.

Sincerely,

Danielle A. Larochelle
Registration Product Manager

Corr. # daL080-04

Bayer CropScience

VOLUME 1 of 2

TRANSMITTAL DOCUMENT

Carbaryl
EPA Registration Number 264-324

Submission in Support of the Reregistration of the Liquid Broadcast Use of Carbaryl on Residential Lawns

Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
Phone: 919 549-2000

Transmittal Date

September 10, 2004

Company Official: _____

Company Name: Bayer CropScience

Company Contact: Danielle A. Larochelle

Telephone: (919) 549-2718

Bayer CropScience
P.O. Box 12014; 2 T.W. Alexander Drive
Research Triangle Park, NC 27709

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P.O. Box 12014; 2 T.W. Alexander Drive
Research Triangle Park, NC 27709

BIBLIOGRAPHY OF SUBMITTED REPORTS

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Volume 2 of 2: Ross, J., J. Driver and C. Lunchick. (2004) Application of Carbaryl Pharmacokinetic Data in the Estimation of Potential Post-Application Health Risks Associated with Broadcast Lawn Care Products. Non-Guideline Document. infoscientific.com, Inc. Project Identification No. is.c 04 Bayer101. Document No. B004716. September 8, 2004. 40pp.

MRID # _____

STUDY TITLE:

APPLICATION OF CARBARYL PHARMACOKINETIC DATA IN THE ESTIMATION OF POTENTIAL POST-APPLICATION HEALTH RISKS ASSOCIATED WITH BROADCAST LAWN CARE PRODUCTS

DATA REQUIREMENT:
Not Applicable

AUTHORS:
John Ross, Ph.D., D.A.B.T.
Jeffrey Driver, Dr.P.H., D.A.B.T., M.T., C.L.S.
infoscientific.com, Inc.

Curt Lunchick, M.S.
Bayer CropScience

STUDY COMPLETED ON:
September 8, 2004

FINAL REPORT

SPONSOR:
Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

PERFORMING LABORATORY:
infoscientific.com, Inc.
Manassas, VA Henderson, NV Sacramento, CA

PROJECT IDENTIFICATION:
is.c 04 Bayer101

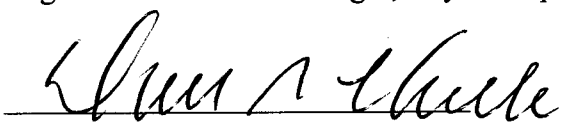
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this document on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

Company: Bayer CropScience

Company Agent: Danielle A. Larochelle

Title: Registration Product Manager, Bayer CropScience

Signature: 

Date: September 9, 2004

Claim of Ownership

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following exposure assessments are not subject to the principles of 40 CFR 160, GOOD LABORATORY PRACTICE STANDARDS (FIFRA), as promulgated in Federal Register, 54, No. 158, 34067-34704, 17 August 1989. Several studies used as references for this document were conducted in accordance with the appropriate GLP standards as verified by the GLP compliance statements found in those study reports.

SPONSOR
Bayer CropScience

Danielle A. Larochelle: _____
Registration Product Manager
Bayer CropScience

Date: 9/9/2004

REPORT AUTHOR

Jeffrey H. Driver: _____
Principal, Infoscientific.com, Inc.

Date: 9/8/04

SUBMITTER
Bayer Crop Science

Danielle A. Larochelle: _____
Registration Product Manager
Bayer CropScience

Date: 9/9/2004

QUALITY ASSURANCE STATEMENT

REPORT TITLE: APPLICATION OF CARBARYL PHARMACOKINETIC DATA IN THE ESTIMATION OF POTENTIAL POST-APPLICATION HEALTH RISKS ASSOCIATED WITH BROADCAST LAWN CARE PRODUCTS

REPORT IDENTIFICATION: is.c 04 Bayer101

This report was audited and reviewed with respect to the analysis and associated documentation. The information in the report is representative of referenced documents / data sources, and the report contents accurately reflect data from these sources.

Auditor: _____ Date: 9/8/04

Terri Driver, B.S.
Staff Scientist, infoscientific.com, Inc.

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I. SUMMARY

Bayer CropScience has undertaken a series of pharmacokinetic studies with carbaryl to characterize and quantify carbaryl-related tissue levels in rats as a function of time following oral, dermal, intravenous and multi-route (oral and dermal) dosing. Results of those studies have direct application for interpretation and estimation of potential human health risks associated with carbaryl exposures, e.g., potential health risks associated with reentry exposures to children following broadcast application to residential turf. This report represents a refined lawn care reentry exposure and risk assessment based on the results of the carbaryl pharmacokinetic studies. Further, as the carbaryl studies are finalized, and additional pharamacokinetic modeling efforts evolve (i.e., model development by Dr. Rory Connolly, CIIT Centers for Health Research and by Dr. Curt Dary et al., EPA, Office of Research and Development), it is anticipated that this report will be edited to include the final results of these efforts. The risk assessment refinements presented in this report are related to a more accurate determination of the biologically effective No-Observed-Adverse-Effect-Level (NOAEL) for cholinesterase-related toxicity, and the absorption, distribution, metabolism and elimination kinetics associated with carbaryl exposures and resulting absorbed dose levels experienced by humans (e.g., children). It is known that rats and humans are very similar with respect to carbaryl-related metabolism and the time course of carbaryl excretion following exposure. Additionally, the pharmacodynamics of carbaryl in rats and humans are very similar as reflected in their respective half-lives for cholinesterase inhibition *in vivo*. The cholinesterase inhibition associated with carbaryl is reversible and of short duration so that there is no "carry-over" of either dose or effect from one day to the next, even if dosing occurs on a daily basis. Further, the scientific weight-of-evidence supports the use of an uncertainty factor less than 100-fold, based on inter- and intra-species consistency in both pharmacokinetics and pharmacodyanamics.

In contrast to the "traditional" method for derivation of the Margin of Exposure (MOE), i.e., NOAEL (mg/kg/day) divided by the absorbed daily dose (mg/kg/day), we describe in this document a refined approach to estimating a biologically effective MOE. This is based upon knowledge of carbaryl's site of action (brain) and the results of the recently-conducted pharmacokinetic studies that quantify brain levels of carbaryl as a function of route, dose, and time. Those studies demonstrate reduced brain concentrations disproportionate to dose, allowing for estimation of peak or plateau levels of carbaryl in brain tissue following route-specific exposure levels anticipated during reentry activities on treated lawns. The conclusion developed from application of the carbaryl pharmacokinetic data is that the revised lawn reentry MOEs for children are in excess of 100, i.e., there is reasonable certainty of no harm associated with carbaryl-based broadcast lawn care product use on residential turf at application rates up to 8 lbs a.i./acre. This conclusion is substantiated by consideration of the revised MOE percentiles associated with the carbaryl-equivalent absorbed dose estimates reported in a residential biological monitoring study involving children (4 to 12 years) in Missouri and California who reentered carbaryl-treated residential lawns where application rates ranged from approximately 2 to 20 lbs a.i./acre.

II. INTRODUCTION

Knowledge about carbaryl in particular is influenced by what is known about the pharmacokinetics and dynamics of other carbamate inhibitors of cholinesterase. A large group of cholinesterase-inhibiting carbamates have undergone various phases of clinical trials in humans as treatments for Alzheimer's dementia such as gangstigmine (Jhee et al., 2003), rivastigmine (Giacobini et al., 2002), heptylphysostigmine (Unni et al., 1994) and eptastigmine (Sramek et al., 1995). Physostigmine, the oldest cholinesterase-inhibiting carbamate that continues to be used in treatment of glaucoma was the prototype compound tested for Alzheimer's (Thal et al., 1983). All of these pharmaceutical compounds were chosen because they have a much longer half life and thus time of effect in humans than carbaryl. Coincidentally, all of these compounds are also more potent inhibitors of cholinesterase than carbaryl.

It is well known that carbamates are quickly metabolized and that cholinesterase inhibition is rapidly reversible, unlike the organophosphates (Ecobichon, 2001). There are numerous examples of a divided dose of carbamate producing less cholinesterase inhibition than the sum of the dosage administered as a bolus. One such example comes from the literature on pre-clinical trials of the Alzheimer treatment candidate heptylphysostigmine (Unni et al., 1994). In that study, young healthy volunteers (n = 21) were administered a single dosage of the compound at 0.6 mg/kg, or a divided dosage (two doses of 0.3 mg/kg/day each). Cholinesterase inhibition between the two dose regimens was then compared. Red blood cell (RBC) cholinesterase was inhibited ~56% at 0.6 mg/kg, but only 10-15% with the 0.3 mg/kg x 2 dose regimen. In addition to pharmaceuticals, there is a wealth of information about carbamate insecticides that have been tested in animals and humans for pharmacokinetics and pharmacodynamics. Pesticides with very short half-life (e.g., carbamates) that have reversible toxicological effects should be tested in a manner commensurate with exposure duration and frequency.

A dose of propoxur that produced evidence of intoxication in humans if given in a single oral bolus caused no observable effects when administered in divided doses spaced half an hour apart (Vandekar et al., 1971). A single oral dose of 0.36 mg/kg of propoxur ingested by human volunteers produced a rapid fall in RBC cholinesterase activity to 57% of control levels within 10 minutes, returning to control levels by 3 hours (Vandekar *et al*., 1971). At 15 to 20 minutes post-ingestion the subjects experienced short-lasting stomach discomfort, blurred vision, moderate facial redness and sweating. However, when propoxur was given as 5 oral doses of 0.2 mg/kg/dose over a 2.5 hour period (at half-hourly intervals), there were no cholinergic signs. Red blood cell cholinesterase activity was depressed to a maximum of 40% of control levels, returning to control levels within 2 hours. The acute no-observed-effect-level (NOEL) in humans for cholinergic signs arising from a single bolus dose of propoxur was 0.2 mg/kg. The cumulative No-Observed-Effect-Level (NOEL) associated with the 2.5 hour (150 minute) repeat dosing (repeat bolus doses) interval, with respect to cholinergic signs in humans, was 1.0 mg/kg. Also, rats exposed daily to a diet containing levels of propoxur at doses estimated to be

222-293 mg/kg exceeding the bolus oral LD_{50} (60-150 mg/kg) exhibited no excess mortality for two years on this regimen (CDPR, 1997).

Another example is oxamyl. In this case the subchronic oral cholinesterase inhibition NOEL is 21-fold higher than in the bolus acute neurotoxicity study, partly because the rats consumed the oxamyl-treated food at night over an extended period, but were not tested for cholinesterase inhibition until the morning (EPA, 1999a). Krieger et al. (1998), conducted studies with methomyl and showed that cholinergic effects were totally absent 1-2 hours following acute intravenous intoxication in the horse showing the rapid reversibility of effect without any medical intervention.

Other examples of repeated administration of a carbamate have occurred with the theoretical exposure extrapolated from a single dose in humans for aldicarb (Tobia et al., 2001). Further examples of the principle can be seen in the clearly defined effect versus no-effect level of methomyl in grape girdlers (O'Malley, 1990) where the difference between an acutely toxic dose and one producing no evidence of effect in a large worker cohort was 3-fold. These data point to the fact that carbamates are readily and quickly detoxified and a dose acquired over several hours is not toxic like a bolus would be.

In addition to the rapid metabolism, the inhibition of cholinesterase produced by carbaryl is readily reversible. In humans and rats the half-life for cholinesterase inhibition *in vivo* is 2.6 and 3.0 hours, respectively (Ross and Driver, 2002). Metabolic profiles in rats and humans are similar in that they produce qualitatively the same major metabolites, i.e., hydrolysis of the carbamate linkage predominates and ring hydroxylation of intact carbamate occurs in both species to a lesser degree. Thus, rat toxicological endpoints and site of action are valid surrogates for humans. The metabolism, pharmacokinetics and pharmacodynamics of carbaryl require unique consideration for risk assessment, and as a result, the default method of estimating Margin of Exposure (MOE) is not appropriate. Sufficient inhibition of brain cholinesterase results in adverse effects (Ecobichon, 2001). Cholinesterase inhibition can be most closely associated with peak or plateau tissue levels in target tissues such as brain (Somani and Khalique, 1987; Kosasa et al., 2000). This suggests an alternative to the classic MOE calculation where total daily dosage at the NOAEL is divided by total absorbed dose estimated for a given human cohort (e.g., child reentry absorbed daily dose associated with dermal and incidental ingestion exposures). The alternative is to estimate the ratio of the peak brain level at the oral, systemic absorbed dose NOAEL (i.e., 1 mg/kg/day) to the peak (following one exposure event) or plateau (following repeat exposure events) brain levels estimated for relevant human absorbed dose levels. While pharmacokinetic data are not available in humans to the extent that they are in rats, given that the available data demonstrate that carbaryl rat and human metabolism and cholinesterase inhibition kinetics are very comparable, we can use the more detailed rat pharmacokinetic data in a surrogate manner for estimating human tissue (i.e., brain) concentrations at the NOAEL and at human-relevant exposure and dose levels.

III. EVALUATION OF PHARMACOKINETIC DATA

The primary route of reentry *exposure* to adults or children following residential lawn care broadcast application of carbaryl is dermal, but two factors mitigate against the dermal route contributing to significant absorbed dose and associated tissue levels *in vivo*. First, dermal exposure is experienced as the result of a series of body-part-specific (bare skin and/or clothed areas) contacts with the treated surface, i.e., turf. The nature of these contacts has been characterized with observational studies (e.g., videotaping) of children's activities and quantifying each discrete dermal contact as the cumulative source of potential dermal exposure. Thus, intermittent dermal exposure is observed to occur over an extended time period (ORETF, 2003a; ORETF 2003b). The EPA's National Human Activity Pattern Survey (NHAPS) results suggest that in the case of children, 75% of the population spends two hours or less per day on turf. Secondly, via the carbaryl pharmacokinetic studies, it has been observed that following a single dermal application to rats, peak blood levels of total ^{14}C-labeled carbaryl occurs approximately four hours after dosing (Krolski et al., 2004a, 2004b). This contrasts with peak blood levels observed in less than 15 to 30 minutes following oral dosing in rats (Krolski et al., 2004a, 2004b).

It is well known that human skin, (i.e., the stratum corneum), provides a very effective barrier to the absorption of many chemicals (Maibach and Feldmann, 1974). The process of percutaneous absorption, largely dictated by Fick's Lawn of Diffusion, effectively results in a lag time for uptake in the bloodstream and thus, "spreads out" the absorption over a longer period than following oral dosing. In the case of carbaryl, this allows for increased opportunity for hydrolysis by enzymes in the dermis, plasma, red blood cells, liver and other organs. The combination of intermittent exposure (multiple contact events over time) in conjunction with slow absorption and multiple sites of metabolism provide greater opportunity for *in vivo* hydrolysis, such that there is likely limited parent carbaryl available for binding to cholinesterase in brain tissue during and following dermal exposure to carbaryl. In rats, following dermal dosing at 20 mg/kg (the approximate rat NOAEL for brain cholinesterase inhibition), the carbaryl concentration in brain was <0.0016 ppm (below the limit of quantitation), while the brain concentration following an oral dose of 1 mg/kg/day (oral NOAEL in rats) was 0.0777 ppm (Krolski et al., 2004a). Thus, the peak brain level at the dermal NOAEL of 20 mg/kg is >48-fold lower than the peak brain level measured after an oral NOAEL dosage of 1 mg/kg. This clearly indicates that while total absorbed dosage may be comparable between routes over time (0 to 4 hours), the peak tissue concentration will be much lower following dermal exposure and the dermal route will provide < 2% of the "brain exposure." Table 1 presents the peak tissue concentrations for total ^{14}C Total Radioactive Residues (TRR) following dermal and intravenous dosing compared to (as a percentage of) peak tissue levels from oral dosing. Note that the ^{14}C TRR in brain are approximately 9-fold higher following oral vs. dermal dosing, while the difference in parent carbaryl levels are 48-fold.

Table 1: Peak Total ^{14}C Equivalent Tissue Levels (TRR) As Percent of Oral Peak Tissue Level

	Whole Blood		Plasma		RBC		Brain	
	LD*	HD**	LD	HD	LD	HD	LD	HD
Dermal	7.7	7.4	10.2	9.0	10.1	3.7	8.9	2.3
IV	139.2	253.6	147.8	185.7	242.9	393.0	587.4	670.7

*LD = Low Dose; 1 mg/kg for IV (intravenous) and oral (gavage); 20 mg/kg for dermal route
**HD = High Dose; 10 mg for IV and oral (gavage); 100 mg/kg for dermal route

Regardless of tissue or dose level, the peak levels following dermal dosing are typically <10% of the peak levels following a comparable oral dosage. This means that for the same absorbed dose, dermal exposure provides only $1/10^{th}$ of the peak tissue levels that oral exposure will. This is consistent with the dermal absorption of 12.7% in the rat which applied to a dermal dose would give a little over $1/10^{th}$ peak oral plasma levels if absorption were instantaneous. As one would expect, peak plasma levels following IV dosing were consistently greater than oral.

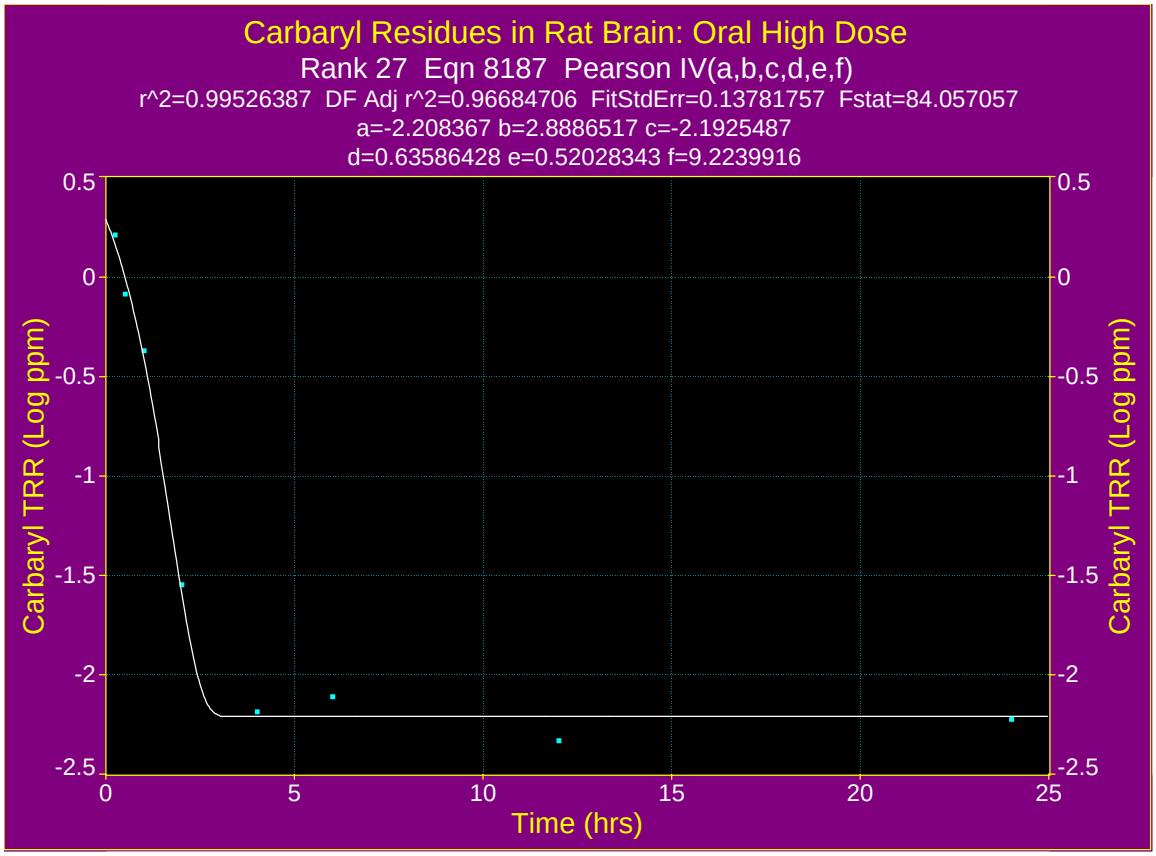
We concentrated our efforts on evaluating the carbaryl kinetics in the alpha (absorption and distribution phase) because it typically lasted for 1.5-2.5 hours in the brain (see Figure 1) which is the time period of interest for exposure to children on treated lawns (EPA, 2001). Further, concentrations of carbaryl could be quantified during this time interval while they frequently dropped below detection limits at or before the beta phase (the shallower slope in Figure 1 corresponding to the elimination phase). In addition, because carbaryl is rapidly metabolized and cleared from the body (see Table 2 below; Ross and Driver, 2002), and the interaction with cholinesterase is readily reversible, only acute exposure and dose resolution is toxicologically relevant. There is no expected "carry over" of either AChE-related effects or parent compound from one day to the next.

Table 2: Comparison of Pharmacokinetic Parameters between Rats and Humans

Species	Plasma $t_{1/2}$ (hr)	Plasma Clearance (L/kg/min)	AChE $t_{1/2}$ (hr)	Dose Excreted as 1-Naphthol (%)
Rat	1.22[a]	0.046[a]	3.0[b]	38[c]
Human[d]	0.79	0.070[e]	2.6	41[f]

[a] From Houston et al., 1974
[b] From Brooks and Broxup, 1995 (see Figure 2)
[c] From Totis, 1997
[d] From May et al., 1992
[e] Obtained by dividing the clearance in L/min by the default male body weight of 77 kg.
[f] Lynkins 1976; Rice 2002a, 2002b; Ross and Driver 2002

Figure 1: Plot of Log Carbaryl Brain Concentration as a Function of Time Following a 10 mg/kg Oral Dose and Illustrating the Alpha and Beta Phase



Shown in Table 3 is the half-life for alpha phase dissipation of total ^{14}C TRR (and carbaryl residues at the high dosages) in various key tissues as a function of oral and intravenous dose. These numbers are remarkable for several reasons. First, the half-life in a particular tissue is consistently shorter at 1 mg/kg than at 10 mg/kg. This indicates that there is faster distribution or metabolism at lower dosages. Secondly, the half-life in tissues following IV is ≤ the oral half-life. This suggests that metabolism by the gut is not the primary determinant of dissipation. Finally, the half-life for actual carbaryl residues (available for only a few tissues) is always less than the half-life of total ^{14}C residues indicating that metabolites have a longer residence time than the parent compound in tissues. It was not possible to estimate half-life for carbaryl itself in the alpha phase following oral dosing at 1 and 10 mg/kg because it dissipated so rapidly, and levels beyond the peak could not be reliably quantified using the standard flow cell radiometric detector. However, at 0.084 mg/kg oral dose a different detection method was employed (fraction collector), and it was possible to estimate half life. All half lives were estimated directly from log linear plots of the tissue concentration as a function of time.

Table 3: Metabolism/Distribution Phase Half-Life Declines with Decreasing Dose

	Total ^{14}C Half-Life (hours)			
Tissue (Route)	**HD (10 mg/kg)**	**LD (1 mg/kg)**	**Ratio LD[a]/HD[b]**	**0.084 mg/kg**
Plasma (Oral)	1.5	1.2	0.80	
Plasma (IV)	1.8 (0.40)[c]	1.2	0.67	
Plasma (Mixed)				0.50[d]
RBC (Oral)	1.2	0.75	0.63	
RBC (IV)	1.0	0.6	0.60	
RBC (Mixed)				0.80[d]
Brain (Oral)	0.8 (0.30)[c]	0.35	0.44	
Brain (IV)	0.6 (0.45)[c]	0.25	0.42	
Brain (Mixed)				0.33[d]

[a] HD = high dose
[b] LD = low dose
[c] Half-life of actual carbaryl residues in the alpha phase
[d] Values were measured following the second dose from a study where rats received two oral doses of 0.084 mg/kg, one hour apart (at time 0 and 1 hr), and a dermal dose of 0.865 mg/kg at time 0, which remained on the rats skin for 2 hours; this is referred to as the "mixed" dose study

The initial plasma concentration of total ^{14}C observed at the earliest time points following dermal application are perhaps the best indicators of what carbaryl concentration at peak might approach at the upper bound. However, this would be only a very crude estimate of plasma level and cannot be used in risk assessment. Carbaryl was not found in plasma following low or high dose oral or dermal dosing at any time point post dosing (Krolski et al., 2004a). It was found transiently at the low and high dose in brain tissue following oral and intravenous dosing (Table 4). The carbaryl in brain tissue was probably due to parent that escaped first pass metabolism in the liver or blood (i.e., at earlier time points than 15-30 minutes) that then was non-specifically partitioned into the fatty tissue in brain, since the K_{ow} shows that carbaryl favors lipid over water. Carbaryl can be measured at low levels in the brain at the NOAEL, but measuring 100-fold below that to reach the regulatory endpoint in the Reregistration Eligibility Decision (RED) document is difficult because it approaches the detection limit of the most sensitive method. In addition to the route effect on peak tissue levels, there is also a dose effect that is disproportionate to the absorbed dose (Table 4). There is a clear change in the rate of metabolism and distribution (alpha phase slope of the dissipation curve) as a function of dose.

These observations make it clear that it is peak (single dose) or plateau (repeat doses, i.e., repeat child hand to mouth-related incidental ingestion events, 20 per hour, during 2 hours of reentry exposure) tissue concentration and not area under the curve (AUC) that determines biological activity of carbaryl. It is also clear that it is not possible to accurately measure parent carbaryl in plasma or other tissue levels at the children's exposure level from direct measurement, so they must be estimated using pharmacokinetic modeling methods.

Table 4: Ratio of Carbaryl Concentration at Peak vs. Total ^{14}C

Tissue/Route	**Peak (hr)**	**Peak TRR (ppm)**		**Peak Carbaryl (ppm)**		**Ratio Carb/TRR**	
		HD*	LD**	HD*	LD**	HD*	LD**
Plasma (Oral)	0.25	7.19	1.44	ND	ND		
Plasma (IV)	0.083/ 0.33[a]	14.32	2.13	3.41[a]	0.085	0.29	0.04
Plasma(Dermal)	4/12[b]	0.69	0.15				
RBC (Oral)	0.25	2.56	0.44				
RBC (IV)	0.083	10.18	1.06				
RBC (Dermal)	4/12	0.095	0.044				
Brain (Oral)	0.25	1.97	0.125	1.63	0.077	0.83	0.62
Brain (IV)	0.083	13.2	0.736	11.8	0.574	0.89	0.78
Brain (Dermal)	4/12	0.045	0.011				

[a] Peak TRR plasma level at high IV dose was 0.33 hours post dosing; whereas, peak carbaryl plasma level at high IV dose was 0.083 hours (5 min) post dosing

[b] Peak plasma, RBC, and brain levels at dermal high dose was 12 hours post dosing

*HD = High Dose; 10 mg for IV and oral (gavage); 100 mg/kg for dermal route

**LD = Low Dose; 1 mg/kg for IV (intravenous) and oral (gavage); 20 mg/kg for dermal route

We have verified that there is little degradation of carbaryl in whole blood during sample collection and subsequent handling prior to analysis. This was potentially a concern in measuring both absolute carbaryl concentrations at low dosages and in estimating half lives in plasma and other tissues. However, as data in Table 3 of Krolski et al., 2004b clearly shows, less than 2% carbaryl was lost from plasma derived from whole blood during ~1/2 hour at room temperature. This time interval is critical because it represents the amount of time the blood may have been at room temperature during the initial collection of blood and centrifugation, and subsequent freezing and thawing prior to chromatography. Losses did not increase significantly with time, and after 24 hours storage were <10%. Thus, plasma levels (or more precisely the lack of measurable parent carbaryl in plasma) are likely not artifacts of sample preparation and this is reaffirmed by the apparent lack of degradation in brain samples, i.e., the percent carbaryl relative to TRR remained very high indicating that loss was due primarily to re-equilibration with blood rather than hydrolysis *in situ*.

If one considers the amount of carbaryl in the brain at the 2-hour time point as "time zero", then the amount in the brain at later time points reflects the total depletion through diffusion or active transport, and degradation. However, there appears to be very little degradation within the brain as the qualitative nature of all extracts (at least at the early time points) is similar (through 2 hours post dosing carbaryl represents >80% of residues in brain). Assuming that degradation within the brain is slow compared to transport (active + passive) out, kinetic analysis can give an estimate for a combined rate constant for transport since we know the absolute value for the amount of carbaryl at each time point. As there is no measurable carbaryl available in the blood after the 2-hour time point to replenish the brain, and any carbaryl that leaves the brain is immediately removed permanently by the blood/liver, we have effectively isolated the

one-way transport system out of the brain. Using the estimate of the "transport out" rate constant from the later time points, one could compare that to the "transport out minus transport in" rate constant from the earlier time points where there is carbaryl available in the plasma. The rate constant for "transport in" can then be calculated (given known values for the other three variables: concentration of carbaryl in brain, concentration of carbaryl in plasma, and the rate constant for transport out). If the "transport in" and "transport out" rate constants are the same, and the rate is first order, then diffusion is probably the mode of transport. If the rates are different, or second order, then transport is likely active. A similar analysis could be done for 1-napthol. There does not appear to be any significant conversion of carbaryl to 1-napthol in the brain, as the relative amounts of the two components in brain stays fairly constant as a function of time at a particular dose.

Carbaryl selectively partitions into lipid. As a result, there are 27 ppm (peak concentration) in fat and 11.8 ppm (peak concentration) in the brain at 5 minutes after an intravenous dose of 10 mg/kg. Compare this to the peak concentration in plasma at 5 minutes (3.4 ppm) and selective lipophilic partitioning becomes clear. This is consistent with the octanol/water partition coefficient of carbaryl (228). In part, this explains why there is measurable carbaryl residue in brain following a low oral dose of 1 mg/kg (0.077 ppm at 15 minutes) while there is no measurable carbaryl in plasma. If the plasma to brain ratio from intravenous high dose (3.4 ppm/11.8 ppm) were operational at the low oral dose, then the estimated plasma concentration following low oral doses would be (3.4/11.8)(0.077) = 0.022 ppm.[1] This is reasonable because the peak carbaryl plasma concentration following low dose I.V. is 0.085 ppm at 5 minutes post-dosing.

There appears to be no change in the relative rate of formation of oxidative versus hydrolytic metabolites with changes in dosage; this clearly indicates that at LOAEL dosages of carbaryl, the predominant pathway is hydrolysis of the carbamate linkage, although there is a small and insignificant amount of oxidative metabolism on the ring while the carbamate linkage is intact. When we look at the metabolic profile at “low” dosages, (more comparable to those experienced by humans), the primary pathway is overwhelmingly hydrolytic. For example, at both the LOAEL and NOAEL in the rat (10 and 1 mg/kg, respectively), the primary metabolite seen at peak plasma concentration of total radiocarbon is 1-naphthol. The other carbaryl metabolites retaining the carbamate linkage (3 and 4-hydroxy carbaryl) represent a total of less than 1 % of the TRR in any tissue (Krolski et al., 2004a) and are also poorer cholinesterase inhibitors than carbaryl (Kuhr, 1971). Additionally, hydroxylated metabolites exist almost exclusively as their secondary metabolites (i.e., glucuronide and sulfate conjugates) that are much more water soluble and even less likely to interact with cholinesterase because of this water solubility and increased molecular bulk.

Brain concentration of carbaryl following two repeat oral dosages of 0.084 mg/kg (at 0 and 1 hr) with concurrent dermal dosage of 0.865 mg/kg (0 to 2 hrs application period) appeared to peak near 15 minutes following the second dose at 0.0045 ppm. Under this

[1] Carbaryl concentration plasma high dose I.V. / brain high dose I.V. = **plasma low dose oral** / brain low dose oral; or carbaryl **plasma low dose oral** level (ppm) = (3.4/11.8)(0.077) = 0.022 ppm.

dosing regimen, the brain level declined to 0.00044 ppm at 3 hours post dosing. A slight increase (0.00064 ppm) was observed at 5 hours probably representing further contribution to brain tissue levels via the dermal route.

Having three different experimentally measured points that relate carbaryl oral dosage in the rat with carbaryl peak brain levels allows meaningful interpolation to lower dosages that might be experienced with children's incidental ingestion or hand to mouth (HTM) exposures (Table 5). Both TRR and carbaryl residues in the brain drop faster than the dosage based on the slopes associated with log brain concentration versus log carbaryl dosage plots. Both carbaryl and TRR concentrations in the brain following a single HTM dose can be estimated from interpolation of a three point graph of brain residue as a function of dosage. The single HTM dosage targeted for interpolation was (0.15 mg/kg)/40 = 0.00375 mg/kg. This discrete HTM dosage represents $1/40^{th}$ of the daily absorbed dose based on the default assumption for HTM frequency and duration in EPA's SOPs (Smegal et al., 2001).

Table 5: Peak Rat Carbaryl Concentration in Brain as a Function of Dose

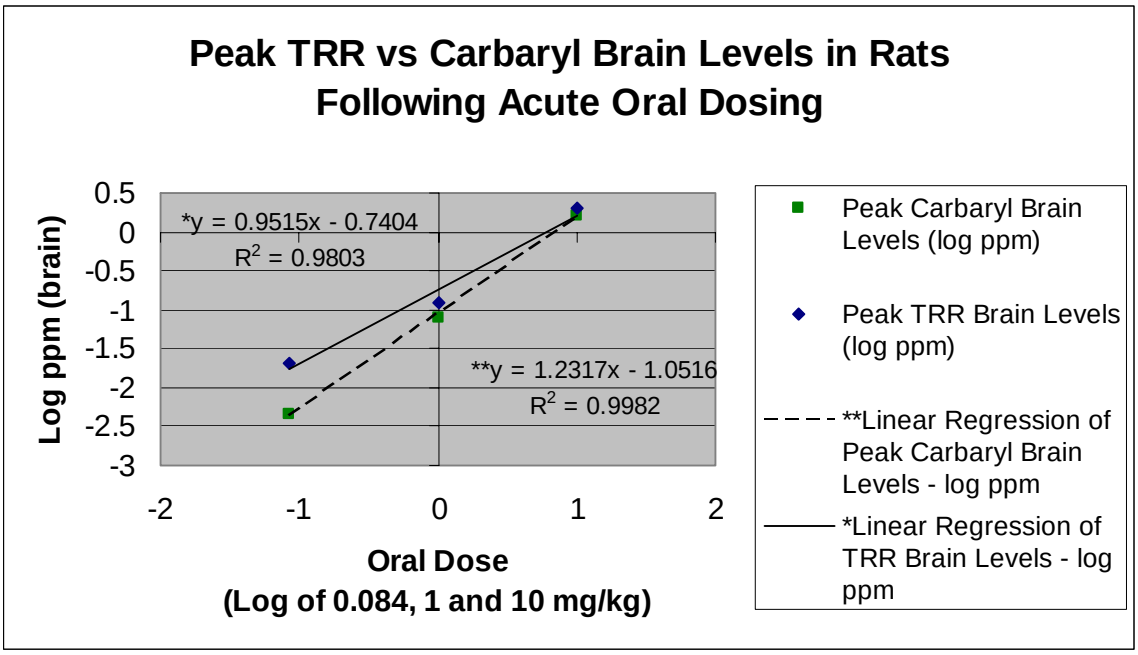
Data Source	**Dose (mg/kg)**	**Log Dose**	**Peak Brain Conc. (ppm)**	**Log Peak Brain Conc.**
Hi Dose Oral	10	1	1.63	0.212
Low Dose Oral	1	0	0.077	-1.114
Mixed Dose Oral	0.084	-1.076	0.0045	-2.347
Interpolated	0.00375	-2.426	0.000091	-4.041

Based on the three point interpolation from data shown graphically in Figure 2 of brain carbaryl concentration versus dosage, the carbaryl brain concentration near peak (15 minutes post oral dose) at an estimated HTM dose of 0.00375 mg/kg was estimated to be 0.000091 ppm. The TRR was plotted similarly (Figure 2) and at a dosage of 0.00375 mg/kg the estimated total radioactive residues were 0.00089 ppm. Interestingly, the two curves (carbaryl and TRR as a function of dosage) diverge over an order of magnitude of dosage based on comparison of the slopes in Figure 2. This suggests that carbaryl levels decline disproportionately to the total TRR in the brain which is curious, since at a given dosage, carbaryl levels closely mirror TRR levels over the time course of several hours. Based on the linear regression the relationship between peak carbaryl brain tissue concentrations and the dose can be quantified as Equation [1]:

$$\mathbf{y = 1.23x - 1.0516} \qquad \mathbf{[1]}$$

where, y is the log of the carbaryl brain concentration in ppm and x is the log of the oral dose in mg/kg.

Figure 2: Peak Carbaryl Brain Levels in Rats Following Acute Oral Dosing: Measured and Interpolated



One might ask why it was necessary to estimate the peak brain concentration at 0.00375 mg/kg (the estimated single HTM dosage) or alternatively why rats couldn't have been dosed 20 times per hour to simulate children's hand to mouth rate. The answer to both questions involves limitations of the experimental method. A single oral dose at 0.00375 mg/kg would yield brain concentrations several fold less than the lowest detection limit. Attempting to orally dose a rat 20 times per hour is extremely traumatic to the animal (which would affect the results and not be allowed by the American Association for Accreditation of Laboratory Animal Care), and would have required several people to dose four rats at this frequency resulting in additional experimental (interperson) uncertainty.

IV. DERIVATION OF MARGINS OF EXPOSURE: ORAL

At some absorbed dose (mg/kg) there will be a No-Observed-Adverse-Effect-Level (NOAEL) for brain cholinesterase inhibition and this will be associated with a particular concentration of carbaryl in the brain (i.e., carbaryl reaches the brain, but at a concentration less than the effective dose). At some lower dose there will be no measurable carbaryl that reaches the brain (i.e., the esterases in the gut wall, plasma, red blood cells, and liver hydrolyze carbaryl before it can reach the brain). At relatively high dosages it appears that the liver is the primary determinant of carbaryl levels, because the concentration of intact carbaryl in the liver is quite high (Krolski et al., 2004a). Oral exposure has much greater potential to achieve a measurable brain level than dermal exposure. If the dosing interval is less than the half-life in tissue, then tissue concentrations will tend to increase to some "plateau". An oral dose less than the NOAEL resulting from successive hand to mouth (HTM) events [e.g., 20 events per hour versus the same dose compressed into 2 events (oral gavage) within one hour as administered in the mixed dose carbaryl "HED Residential SOP toddler dose simulation" study in rats] will likely produce carbaryl plateau levels in the brain somewhere near or below the detection limit. We know this because at an oral dose of 1 mg/kg, carbaryl brain levels are more than 20-fold less than at the 10 mg/kg despite only a 10-fold dose difference.[2] As a result, it is necessary to model carbaryl levels in the brain following an exposure magnitude and frequency relevant to children during reentry of treated lawns to estimate a Margin of Exposure (MOE). The estimated brain levels and MOE are conservative because the reduction in dose from NOAEL produces a disproportionate reduction in brain concentration. The disproportionate reduction is evident from several methods of estimation. First, we know that the ratio of brain to plasma concentration at peak for IV high dose vs. IV low dose is 21.0 and 20.6, respectively. At lower dosages encountered by a child, the effect will be at least proportional to dose but likely even greater. Therefore, it is important to estimate brain plateau concentrations after divided oral HTM doses and from those brain concentrations, estimate a refined MOE.

Given that peak brain levels associated with dermal exposure are significantly lower than peak brain levels associated with lower oral doses and in addition, are delayed by several hours relative to peak levels following oral dosing as demonstrated in the carbaryl rat pharmacokinetic studies, the refined or revised MOE calculations focus on the oral route.

The traditional MOE for children's exposure is calculated as follows in Equation [2]:

$$\textit{MOE} = \textit{oral NOAEL/oral HTM dosage} \quad [2]$$
$$= \textit{(1 mg/kg/day)/(0.25 mg/kg/day) = 4}$$

Where, 1 mg/kg/day is the oral NOAEL, and 0.25 mg/kg/day (0.15 mg/kg was estimated to be the oral route contribution) is the total absorbed dose level estimated using methods recommended in EPA's Residential Exposure Assessment SOPs (EPA 1997, 1999b) as

[2]This relationship continued in the mixed dose study where a combined initial time 0 oral dose of 0.084 mg/kg and dermal dose of 0.865 mg/kg produced a 17-fold decrease in peak brain carbaryl levels compared to the 1 mg/kg oral dose level while the decrease in the oral dose was less than 12-fold.

described in the carbaryl IRED (EPA 2003a). A very similar result is obtained if we divide the brain concentration at a bolus dose of 1 mg/kg (0.077 ppm) by the brain concentration following a bolus dose of 0.25 mg/kg (0.016 ppm estimated from lower the curve in Figure 1), i.e., 0.077/0.016 = 4.8 MOE. This provides some reassurance that the method of using peak brain concentrations provides a similar MOE when using bolus doses.

However, this method of calculating the MOE assumes that the oral and dermal systemic dosage received by a child occurs as a bolus absorbed daily dose, without consideration of route-specific time dependency. The oral and dermal exposures are experienced in a time-dependent manner. Incidental ingestion related to hand to mouth (HTM) behavior has been observed to be experienced in discrete events, i.e., in the case of children (approximately 1 – 6 yrs), an average of 10 to 20 events per hour, respectively; ORETF, 2003a; Smegal et al., 2001. Further, the time course related to absorption kinetics and associated brain levels following discrete, intermittent oral versus dermal exposures (Krolski et al., 2004a) indicates that the refined or revised child lawn care reentry short-term MOE (in contrast to the traditional MOE shown above) should be calculated using the *measured peak brain level in rats (0.077 ppm) following an oral bolus at the NOAEL (1 mg/kg) divided by the estimated plateau brain level following a divided oral dose relevant to the HTM exposure scenario* (20 events per hour for 2 hours, where the discrete HTM dose is 0.00375 mg/kg per HTM event; i.e., 0.15 mg/kg/day / 40 events in 2 hours per day = 0.00375 mg/kg).

The most appropriate method of estimating the brain concentration from a divided oral HTM dose is the plateau principle. We can estimate the average carbaryl concentration in brain at 2 hours based on its half-life in the brain following a single oral dose at 0.084 mg/kg and using a modified equation for the plateau principle (Mayer et al., 1980; equation [2]). This is based upon the estimated peak brain concentration of carbaryl following a single HTM event and the estimated half-life for loss from the brain, i.e., 0.33 hours (Table 3). It is important to note that the half-life of 0.33 hours was obtained from the mixed-dose study and reflects concurrent oral and dermal dosing; however, the half life of carbaryl in brain following a LOAEL oral dose is also 0.3 hours. If we assume an oral dose is received via HTM every 0.05 hours or every 3 minutes (60 mins per hour / 20 HTM events per hour), and that initial HTM dose (0.00375 mg/kg) produces a carbaryl brain concentration of 0.000091 ppm, the plateau concentration at 2 hours (using a brain tissue carbaryl elimination half-life of 0.33 hours) is 0.0011 ppm. The temporal, stepwise oral exposures, elimination, and plateau profile is illustrated graphically in Figure 3.

Figure 3: Carbaryl Plateau (Peak) Brain Level (0.0011 ppm) Following Discrete HTM Oral Doses of 0.00375 mg/kg (corresponding to 0.000091 ppm in brain tissue) Every 3 Minutes for 2 Hours (Carbaryl brain half-life = 0.33 hrs).

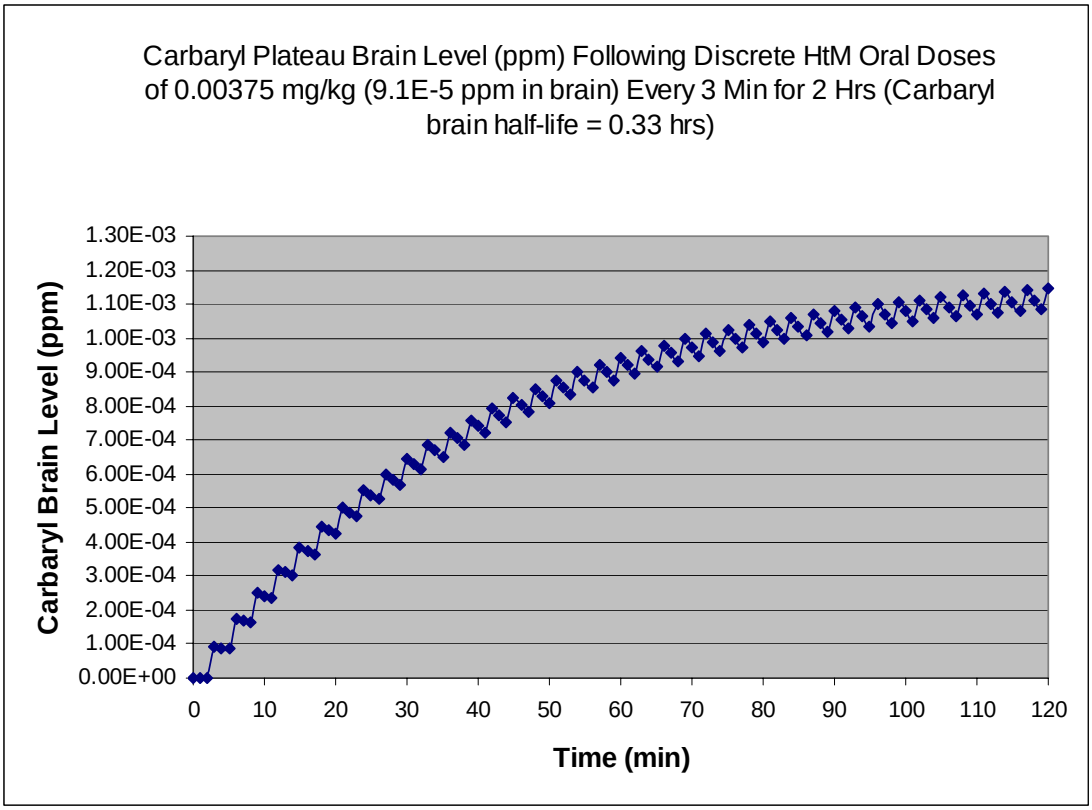


Figure 3 presents the plateau (peak) carbaryl brain level (0.0011 ppm) estimated for the children HTM scenario using the following equation:

$$C_p = (C_{d\_event1} \times F) + (C_{d\_event2\ thru\ n} \times F) \quad [3]$$

Where,

C_p = carbaryl concentration (mg/mL or ppm) in the brain at plateau or peak;
$C_{d\_event1\ thru\ n}$ = concentration (mg/mL or ppm) in the brain (0.000091 ppm) at each incremental time step (min), where an oral dose of 0.00375 mg/kg occurs at a frequency of 20 events per hour or once every 3 minutes (0.00375 mg/kg = 0.15 mg/kg/day / 40 events per day); and
F = fraction of brain level eliminated at each time step (0.025 per min based on 0.5 in 0.33 hrs [the measured carbaryl half-life (hrs) in brain at 10 mg/kg following oral dosing was 0.30 hours and the estimated carbaryl half-life following first oral dose of 0.084 mg/kg and concurrent dermal dose of 0.865 mg/kg was 0.33 hours]). Data from Krolksi et al., 2004b.

The concentration estimated in the brain at plateau following repeated HTM contact is 0.0011 ppm. This estimated concentration can be compared to a measured peak concentration in the rat brain following the second oral dose in the mixed dose study. Fifteen minutes after the second dose (at or near peak concentration), the measured brain concentration was 0.0045 ppm. One would expect the brain concentration at peak following the second oral dose to be larger than if the doses had been divided more finely at smaller intervals. Thus, the measured value is within 4-fold of the value predicted, and provides a degree of confirmation of the estimated plateau level.

The resulting refined child lawn care reentry MOE can then be expressed as:

$$MOE = C_{NOAEL} / C_p \quad [4]$$

$$= 0.077/0.0011 = \mathbf{70}$$

Where,
C_{NOAEL} = peak carbaryl brain level in rats (0.077 ppm) following an oral bolus at the NOAEL (1 mg/kg); and
C_p = estimated plateau or peak brain (0.0011 ppm) level following a divided oral dose relevant to the child HTM exposure scenario for carbaryl.

This MOE is approximately 20-fold lower than that estimated using the traditional MOE approach (70 / 4 = 17.5). This adjustment factor, i.e., 20-fold, can be used refine the traditional MOEs estimated for the carbaryl-equivalent doses derived from Bayer CropScience's residential biological monitoring study.

The traditional MOEs can be increased by a factor of 20 to approximate refined or revised MOEs (Table 6). The revised child MOEs exceed 100 across the distribution of carbaryl total absorbed doses measured in the residential biological monitoring study. The MOE distribution is likely to be conservative given that only a fraction of the total daily absorbed dose probably "originated" from the oral route via incidental ingestion exposure (i.e., the children's total absorbed dose likely included both oral and dermal exposures). It is even more conservative when one considers that the pre-application exposure levels from biomonitoring were not subtracted. These pre-application exposures ranged up to 0.0125 mg/kg in children age 4-12. There are many sources of naphthol other than carbaryl, including ingestion of pre-hydrolyzed carbaryl in food, naphthalene from solvents and gasoline.

Table 6: Carbaryl Equivalent Absorbed Dose and Plateau/Peak-Based MOE Distributions for Children Associated with Lawn Care Reentry.

Percentile	***Carbaryl Equivalent Daily Absorbed Dose*: Children 4 to 12 yrs (µg/kg)*** **(MO and CA** combined); n = 162)**	***Traditional MOE (NOAEL / Daily Dose)***	***Revised MOE (Traditional MOE x 20***)***
0	0.005	200000	4000000
1	0.005	200000	4000000
5	0.005	200000	4000000
10	0.005	200000	4000000
20	0.070	14212	284236
30	0.28	3565	71297
40	0.98	1017	20342
50	1.4	710	14195
60	2.1	465	9310
70	2.9	342	6850
80	5.3	190	3806
90	11.8	85	1695
95	23.7	42	844
97	52.3	19	383
99	68.9	15	290
99.9	117.2	9	171
100	126.2	8	158
EPA SOP	250	4	80

*Based on individual daily dose values, i.e., dose estimates were calculated based on the 1-napthol urinary biological monitoring results for each individual person-day, adjusted to represent recent, within 96-hour, carbaryl equivalent doses; this was accomplished by adjusting the 1-napthol urinary measurements using a stoichiometric conversion factor of 3.5 to account for 1) molecular weight differences between carbaryl and 1-napthol (1.4 = MW carbaryl / MW 1-naphthol = 201.2 / 144.2) and 2) a factor of 2.5, to account for only 40% of a carbaryl dose being eliminated in urine by humans; thus, 1.4 x 2.5 = 3.5 (Ross and Driver, 2002).
**CA cohort data were censored to exclude child dose estimates from residential sites where the reported application rates were greater than 20 lbs a.i./acre (i.e., greater than 2 times the maximum label rate for broadcast turf application of 8 lbs a.i./acre).
***20-fold adjustment factor = Revised EPA SOP HTM Scenario MOE, i.e., 70, divided by the previous EPA SOP traditional MOE, i.e., 4 (70 / 4 = 17.5, rounded to 20).

In EPA's risk assessment for carbaryl, the absorbed dose of "parent" carbaryl from the 1-napthol urinary biomontoring data was calculated in a manner that provided an upper-bound estimate of the absorbed dose. The Agency defined the calculated dose as the "Post Application Total Dose", which represented *carbaryl equivalent dose residues in urine added together from the day of application through the last day of monitoring as the excretion profile of carbaryl/1-napthol indicates a 96 hour clearance interval* (Tables 33 and 34 of the IRED, EPA, 2003a). The Post Application Total Dose estimates for each individual child were calculated by the

Agency in Table 12/Appendix N of the IRED document: Children's Data Analysis – Based on Age. Plateau-based MOEs using the Agency's method of calculating the post application carbaryl dose were also calculated by adjusting the traditional MOE by 20 to correct for the actual peak brain carbaryl tissue levels estimated at the dose levels in the range occurring following lawn application (versus those at the oral NOEL of 1 mg/kg/day). The California cohort data were censored in the Agency spreadsheet to exclude child dose estimates from sites where the application rates exceeded 20 lb a.i./A. The revised MOEs are presented in Tables 7 and 8.

Table 7: Summary Statistics For Children (4-5 years)

Statistic	**Post Application Dose Total Dose (µg/kg/day)**	**Revised MOE (Traditional MOE x 20)**
Average	44.4	450
Minimum	0.6	33,333
Maximum	219.9	91
Median	18.2	1100
25th%tile	11.2	1786
75th%tile	27.9	717
95th%tile	174.1	115
99th%tile	210.7	95

Table 8: Summary Statistics For Children (6-10 years)

Statistic	**Post Application Dose Total Dose (µg/kg/day)**	**Revised MOE (Traditional MOE x 20)**
Average	23.1	866
Minimum	1.0	20,000
Maximum	120.6	166
Median	14.3	1398
25th%tile	7.8	2564
75th%tile	24.3	823
95th%tile	64.7	309
99th%tile	109.4	183

V. SENSITIVITY ANALYSIS

It is important to emphasize that the MOE calculated by EPA in the RED (EPA, 2003a, 2003b) are conservative, i.e., the estimates likely overestimate actual health risks. There are multiple reasons for this overestimation bias as outlined below. EPA's introduction to the Standard Operating Procedures (SOPs) for calculating residential exposure acknowledge that the resulting exposure estimates will be conservative, i.e., the original SOPs were developed using a deterministic, assumptive approach to exposure assessment that intentionally produced bounding estimates or were representative of high-end exposures (EPA 1997, EPA 1999b). EPA has also acknowledged that the procedures outlined in the original document were not meant to be aggregated (e.g., across potential exposures from two or more residential scenarios, and with dietary sources) without a definitive characterization by the assessor because it violates those basic tenants of exposure assessment by adding highly conservative (compounded conservatism) estimates of exposures that result in "bounding, unrealistic estimates of exposure" (EPA 1992, EPA 1999b, Bogen 1994, Burmaster and Bloomfield 1996, Burmaster and Harris 1993, Cullen 1994).

Thus, it is important to characterize sources of uncertainty and their magnitude, and the degree of conservative bias that may exist. In this section we highlight additional factors not incorporated into our analysis that would make the exposure lower and the resulting estimates of safety even higher.

The hand-to-mouth (HTM) exposure assumptions involve multiple input factors that tend to be conservative. For example, hand to mouth frequency is assumed to be 20 events per hour based on the results of a single study (Smegal et al., 2001). However, a summary of additional studies indicates that the average number of hand to mouth events in an hour for children is closer to 10 (ORETF, 2003a). This assumption alone might cut the estimated hand to mouth exposure by half. Additionally, EPA assumed the surface area contacting the mouth to be 20 cm^2. A recent summary of children's hand to mouth activity involving both video and actual anatomic measurements of children's hands indicates that surface area contacting the lips and mouth are approximately 7 cm^2 per hand to mouth event for children 4-5 years old (ORETF, 2003b). One clear source of overestimation is the assumption that contact with lips results in the same degree of removal as insertion of the fingers into the mouth. The assumption is made for simplicity, but the lips do not have the same solvating capacity as the buccal surface. Another very significant assumption is that each contact of hand to mouth is interspersed with the same hand contacting a treated surface. Videography clearly shows that this does not happen, and that hand to mouth events as well as hand to surface events, are typically "clustered". As a result, hands are not fully "loaded" with a chemical when they approach the mouth (ORETF, 2003b). Finally, solvation or removal efficiency of hand residues by mouth contact is assumed to be 100%. This estimate is also an upper bound, although in the case of carbaryl may be closer than many chemicals due to its water solubility.

As a result of the compounded conservatism resulting from use of multiple upper bound assumptions included in hand to mouth-related exposure and dose estimation, the resulting health risk estimates are likely to be conservatively biased (i.e., actual MOEs would likely be higher). We examined the effect of assuming a longer brain half life, i.e., 0.45 hours as measured in the I.V. high dose study in rats (see Table 3), longer duration of HTM activity, the effect of repeated hand to mouth actions or events in a clustering sequence (ORETF 2003a), and the effect of changing the assumed frequency of HTM events (ORETF 2003a) using Equation [3]. Each of these changes would result in increased estimates of brain concentration following an oral dose. In addition, two input variables also used in the EPA SOP methodology for HTM were evaluated, i.e., finger surface area involved (actually inserted in mouth) in HTM behavior and saliva removal efficiency (ORETF 2003b; NDETF saliva removal efficiency studies). Table 9 presents a summary "sensitivity analysis" of the above described variables and their respective impact on predicted carbaryl brain levels.

Table 9: Summary of Iterative Sensitivity Analysis

Variable	Range	Brain Level Effect
Brain half life (hr)	0.33→0.45	Increase 25%
HTM duration (hr)	2→4	Increase 8%
Clustering (clusters x events/cluster)	20 events per hour, evenly spaced versus 4 clusters of 6 events per hour, spaced at 10 minutes[a]	Increase 27%
HTM frequency	20→10	Decrease 46%
Finger surface area inserted (cm^2)	20→7	Decrease 65%
Removal efficiency (%)	100→50	Decrease 50%

[a] Four clusters of six consecutive HTM events per hour, where each cluster is 10 min apart. In contrast, the baseline assessment used the SOP-based assumption of 20 events per hour, evenly spaced, i.e., 3 minute apart.

Predicted brain concentration is affected by the brain half life of carbaryl and the clustering of HTM events. However, it is important to note that the brain half-life value of 0.33 hours is the recommended value because it represents a half life via oral administration, rather than intravenous. Thus, the brain half life of 0.33 hrs is associated with a higher degree of relevance and confidence for oral exposure/dose simulations. Clustering hand to mouth events (i.e., assuming that the hand goes to the mouth several times in succession rather than spread out every 3 minutes) produces a significant increase, but this interpretation of clustering is not very plausible because there would be less opportunity for "reloading" or replenishing of carbaryl residues on the hand from the treated lawn surface between HTM events. This has been demonstrated via repeat hand press studies conducted by the Non-Dietary Exposure Task Force (NDETF) of which Bayer CropSciences is a member. In contrast, to variable changes that increase predicted brain levels, other reasonable changes in input variable values, i.e., HTM frequency, surface area of hands/fingers involved, saliva removal efficiency, decrease the predicted brain level. The alternative variable values used have been documented in publications (e.g., ORETF 2003a, 2003b; HTM frequency on lawns and finger surface area involved in HTM behavior) and GLP-based exposure monitoring studies (NDETF; saliva removal efficiency) as reasonable alternatives to the standard upper-bound values used in EPA's SOP 12.

VI. DERIVATION OF MARGINS OF EXPOSURE: DERMAL

Following a bolus dermal dose, the peak tissue levels are not achieved until 4-12 hours post dosing (see Krolski et al., 2004a). Considering the fact that a human dermal dose will be acquired over a two hour period, this further delays the peak with respect to oral exposure. Because there is a clear separation in time of peak tissue concentrations from oral vs dermal exposure, there will be very little overlap or contribution of dermal dose to oral dose tissue concentrations. This is graphically illustrated in the mixed dose brain concentration data (Krolski et al., 2004b). Following the peak concentration at 15 minutes post oral dosing, the next peak occurs between 3 and 5 hours (2-3 hours after the second oral dose) and this secondary peak results from the dermal component of the mixed dose administration. Thus, we can separately consider the MOE for oral exposure from the MOE for dermal exposure in the context of a concurrent dermal and oral (HTM) child lawn care reentry scenario.

The secondary maximum carbaryl brain level measured in the mixed dose experiment was 0.00064 ppm at 5 hours post dosing. This level was slightly higher than the only previous measurement at 3 hours when brain levels were 0.00044 ppm. The low dose dermal experiment demonstrated that the peak brain levels were achieved at 4 hours post dosing, and the 4-hour level was 19% higher than the level measured at 2 hours (the next highest TRR). We can estimate the peak level in the mixed dose experiment [(0.00064 + 0.19 x 0.00064] = 0.00076 ppm, and using this level, the dermal MOE can be calculated using equation [4].

$$0.077 \text{ ppm} / 0.00076 \text{ ppm} = 101$$

This is a very conservative estimate of MOE for the dermal dose, because it was measured in rats which typically have 5 times the dermal penetration of humans (Ross et al., 2001). As a result, the true MOE from dermal exposure is likely approximately 5-fold higher.

VII. CONSIDERATION OF UNCERTAINTY FACTORS FOR INTERSPECIES AND INTRASPECIES PHARMACOKINETICS

Regardless of whether MOE is calculated traditionally or based upon the proposed ratio of brain levels, the interpretation of MOE is a risk management (policy) decision based upon the scientific weight-of-evidence (e.g., EPA July 1999 Guidelines for Carcinogen Risk Assessment; http://cfpub2.epa.gov/ncea/raf/cancer.cfm). It is typical to apply a default uncertainty factor ("UF") of ten (10) for interspecies variability and also for intraspecies variability. The World Health Organization (WHO 2001), following the suggestion of Renwick (1993), recommends dividing the standard default UF of ten (10) for interspecies variability between a factor of four (4) for toxicokinetics and a factor of 2.5 for toxicodynamics, when adequate data are available to address these factors. Similarly, WHO recommends dividing the standard default UF for intraspecies variability into separate toxicokinetics and toxicodynamics components of 3.2 each, when adequate data are available to address these factors.

The robust carbaryl database shows similar toxicokinetics, including metabolism, in humans and various species of test animals. The available data justify an UF of one (1) or less (out of a possible 4) for the toxicokinetics portion of the UF for interspecies variability. Due to limitations in the available human data on carbaryl toxicodynamics, however, it is appropriate to use the standard default value of 2.5 for the toxicodynamics portion of the UF for interspecies variability. Accordingly, the overall UF of 2.5 for the interspecies variability of carbaryl is appropriate, and the default UF of 10 is unnecessarily high.

The extensive literature on absorption, distribution, metabolism, and excretion of carbaryl in humans (Ross and Driver, 2002) shows moderate differences between individuals or human subpopulations. Consequently, the literature justifies a value of two (2) (out of a possible 3.2) for the toxicokinetics portion of the intraspecies UF. Due again to limitations in the available human data on carbaryl toxicodynamics, the standard default value of 3.2 appears to be appropriate. Thus, an intraspecies UF of 6.4 is justified, while use of the default value of 10 is unnecessarily high.

A combined UF of 16 for interspecies and intraspecies variability (*i.e.*, 2.5 x 6.4) is justified, and the typical default values (10 x 10 = 100) is unnecessarily high, given the abundance of relevant data on the toxicokinetics of carbaryl in humans and animals, as explained in greater detail below. Thus, the recommended combined uncertainty factor for carbaryl is 48 rather than the currently used value of 100.

When there are adequate human toxicology data, there is no need to use an interspecies factor, because there is no uncertainty associated with an extrapolation between species, *i.e.*, from laboratory animal to man. Similarly in those situations where the sensitivity of the test species to the agent is the same as that of humans, the interspecies factor is one. In the case of carbaryl, extensive data in humans are available. Applying criteria developed by a WHO expert group (WHO, 2001), we have carefully examined this database for purposes of determining whether a chemical-specific uncertainty factor for

interspecies pharmacokinetics could be developed for carbaryl in lieu of the default value.

WHO (2001) has recommended that the interspecies uncertainty factor be subdivided into a four-fold component for pharmacokinetics and a 2.5-fold component for toxicodynamics. This recommendation follows the analysis published by Renwick (1993), which shows that a slightly greater interspecies variability can be found in the area of pharmacokinetics than in the area of toxicodynamics. Concordance in the pharmacokinetics of the test agent in animals and man argues strongly for an uncertainty factor of less than ten (10). There are several examples of U.S. EPA reducing the pharmacokinetics portion of the interspecies uncertainty factor to one. For these chemicals, U.S. EPA concluded that the interspecies uncertainty factor could be reduced from the default value, based upon dosimetric considerations in the extrapolation of animal data to humans.[3]

Substantial, available data on carbaryl show a similar pattern of metabolism and of other aspects of carbaryl pharmacokinetics in humans and in various species of laboratory animal. Absorption, distribution, metabolism, and excretion have been well studied in laboratory animals, primarily rats, and knowledge of the pharmacokinetics of carbaryl in humans is available from a number of published studies based upon purposeful pharmacokinetic studies, accidental poisoning from dermal and oral exposures, and monitoring of worker populations. Multiple detoxification pathways exist in animal species, and urinary excretion is primarily in the form of naphthols in both humans and animals. The urine is the primary route of excretion in humans and in laboratory animals after both dermal and oral exposure.

In determining an appropriate uncertainty factor for the interspecies pharmacokinetics of carbaryl, the criteria applied were recommended in a recent draft guidance document developed by a group of international experts under auspices of the WHO (WHO, 2001). These criteria and consideration of them, in light of the carbaryl database, are discussed below.

The extensive available database and the similarity in pharmacokinetics between humans and laboratory animals support the conclusion that there is no need for a value of greater than two (2) for the pharmacokinetics portion of the interspecies uncertainty factor for carbaryl. Accordingly, the default uncertainty factor of four (4) is considered both overly conservative and unnecessary.

In conclusion, the 10X interspecies uncertainty factor historically used is considered to be excessively conservative, given the large body of available toxicity data comparing

[3] *See* U.S. EPA IRIS summaries for the establishment of RfCs for ethyl benzene (www.epa.gov/iris/subst/0051.htm), ethyl chloride (www.epa.gov/iris/subst/0523.htm), 2-ethoxyethanol (www.epa.gov/iris/subst/0513), and acetylnitrile (www.epa.gov/iris/subst/0205.htm). The basis for the reduction by the US EPA of the interspecies uncertainty factors for these chemicals can be found in the document entitled "Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry" (EPA/600/8-90/066F, October 1994).

humans and laboratory animals and the similarities in pharmacokinetics that have been demonstrated in mammalian species. Instead, the default value (2.5X) is recommended for the toxicodynamics portion of the interspecies uncertainty factor for carbaryl, due to limitations in the data available for the assessment of human sensitivity to carbaryl-associated toxicity. There appears to be no justification for an uncertainty factor of greater than two (2) for interspecies variability in pharmacokinetics, considering the previously discussed similarities in pharmacokinetics between humans and laboratory animals.

The WHO's International Program on Chemical Safety ("IPCS") recommends a series of questions to help determine the appropriate interspecies chemical-specific adjustment factor (uncertainty factor) for pharmacokinetics. Below, responses based on the carbaryl database follow each question:

1. Are data on interspecies and intraspecies pharmacokinetic differences available?

Yes. Extensive literature concerning pharmacokinetics in laboratory animals is available, and several publications describe the absorption, metabolism, and plasma and urinary levels of 1-naphthol after occupational and controlled exposures in humans. Most pharmacokinetic data have been collected after dermal or oral exposures. Naphthols are the primary metabolites found in humans, rodents, and other mammals. Humans appear to absorb carbaryl less efficiently by the dermal route than rodents or rabbits. The pattern of excretion and the half-life for elimination are similar in humans, rats and other mammalian species (see Table 2 in Section III and Table 10 below; Ross and Driver, 2002).

Table 10: Summary of Key Carbaryl Metabolism and Pharmacokinetic Studies Reported in the Literature

Reference	Species	Label Site	Dose (mg/kg)	Route[a]	Coll'n Time (hr)	% in Urine	% 1-Naphthol	
							sulfate	glucuron
Best, 1962	Human	N/A[b]	N/A	occup.	grab	N/A	N/A	N/A
Knaak, 1965	Rat	Naphthyl	3	i.p.	24	73[c]	23	16
	Human	N/A	N/A	occup.	24	N/A	16	83
Knaak 1968	Human	N/A	2	p.o.	24	?[g]	9	13[h]
	Monkey	Naphthyl	300	p.o.	48	?	0	0
	Pig	Naphthyl	25	p.o.	120	83	0	6
	Rat	Naphthyl	30	p.o.	24	68	6	8
Feldmann, 1974	Human	?	0.001	i.v.	120	7	?	?
Lykins, 1976	Human	N/A	0.25-1	p.o.	48	49	18	~12[i]
Struble, 1994	Rat	Naphthyl	50	p.o.	168	85	1[j]	15
Totis, 1997	Rat	Naphthyl	50	p.o.	168	86	24	15
Valles, 1999	Mouse	Naphthyl	50	p.o.	168	84	14	17

[a] occup. = occupational exposure of unknown amount during packaging of carbaryl dust; i.p. = intraperitoneal; p.o. = oral; i.v. = intravenous
[b] N/A = not applicable
[c] Percent in urine was reported for oral dose only at 20 mg/kg
[g] Unknown or unstated
[h] Total 1-naphthol was determined by the method of Best and Murray, 1962 and found to be 38% of the dose.
[i] Estimated from percentage of fraction #3 thought to be glucuronide conjugate of 1-naphthol based on the fraction of #3 that could be acid hydrolyzed to 1-naphthol
[j] The report gives contradictory numbers of 1 and 6%.

2. Did the studies use adequate methods (e.g. analytical and appropriate duration)?

Adequate and appropriate methods were used in the available pharmacokinetics studies. Most of the human studies were by the oral route of administration. Limited data are available on occupational exposures involving both the dermal and the inhalation routes of exposure. Pharmacokinetic studies have used radiolabeled carbaryl and standard techniques, such as high pressure liquid chromatography (HPLC), to quantify levels in blood and urine. The analytical methods appear to be adequate and include radiotracer liquid scintillation counting, thin layer chromatography, and HPLC/MS. The controlled oral studies examined single exposures, and the occupational exposure studies were subchronic.

3. Has the active chemical moiety been identified?

Yes, the parent compound is toxicologically active. Ring-hydroxylated metabolites of carbaryl are the only metabolites with cholinesterase-inhibiting potential and are more polar and, thus more readily excreted. Although they also

may contribute to some forms of carbaryl-associated toxicity, these metabolites are known to be less toxic than carbaryl itself (Kuhr, 1971).

4. Is it known whether toxicity depends on AUC or C_{max}?

Yes. It is very clear that with the rapid metabolism of carbaryl, inhibition of cholinesterase is a function of peak tissue levels. Also, the toxicity seen in the critical short term studies is consistent with dependency on the C_{max} (maximum blood/tissue concentrations) rather than the AUC (area under the curve).

5. Is a PBPK model required to describe target organ dosimetry?

No. A physiologically-based pharmacokinetic (PBPK) model for carbaryl has been developed for rats (ORD, 2004) and will be used for simulating human dosimetry underlying varying exposure/dose regimens. The model has not yet been used for risk assessment of carbaryl and is not required, but pharmacokinetics would help to refine the risk assessment of rapidly metabolized, reversible cholinesterase inhibitors such as carbaryl. Another PBPK model is being developed independent of ORD (Connolly, 2004).

6. Are animal and human populations appropriate and comparable?

Yes. Most data in laboratory animals and humans have been developed in healthy adults. More limited data are available for children and young rats. Human pharmacokinetic studies have used only males, but worker biomonitoring studies have included females. Pharmacokinetic studies in laboratory animals have included both males and females. The laboratory animal and human populations are considered to be appropriate and comparable.

7. Was the route of administration appropriate?

See item 2 above. The dermal route is the primary route of occupational and residential exposure, and pharmacokinetic data are available following dermal exposure in humans. Pharmacokinetic information is available in humans following mixed (inhalation and dermal) exposure, and in laboratory animals following oral exposure.

8. Were doses appropriate to expected human exposure?

Dose levels in the pharmacokinetic studies spanned a wide range and were considered appropriate to human exposure, although most of the doses were significantly greater than the average human dose. Dose levels in the human and laboratory animal studies conducted by the dermal route overlapped.

9. Were the number of subjects and samples adequate?

There were 4 rats per dose level/time interval in the dermal absorption/pharmacokinetic study of (Cheng, 1994). Blood and urine levels of carbaryl metabolites were measured in a population of workers (Best and Murray 1962; Knaak et al., 1965). Six human subjects were used in the dermal absorption/pharmacokinetics study of Feldmann and Maibach (1974). Several poisoning case reports provide additional information but are limited to reports about individuals. Blood and/or urine measurements are available from occupational studies of workers involved in the manufacturing of carbaryl (summarized in Ross and Driver, 2002).

Extensive data are available allowing comparison of the time course of absorption, distribution, and excretion between rodents and humans after dermal exposure. Available data, primarily from *in vivo* studies and urinary monitoring, indicate similar metabolism in rodents and humans. These data are sufficient to conclude that similar pharmacokinetics are found in humans and laboratory animals. The primary difference in pharmacokinetics between humans and rodents is that humans absorb carbaryl less efficiently by the dermal route of exposure. Noting the limitations in the available pharmacokinetics data by the inhalation route, we recommend a value of two (2) for the pharmacokinetics portion of the chemical-specific adjustment factor (uncertainty factor) addressing interspecies variability. However, a value of one (1) can be justified for the dermal route of exposure. Therefore, the value of two (2) recommended as the chemical-specific adjustment factor for interspecies pharmacokinetic variability is very conservative.

VIII. CONCLUSIONS

There are many natural sources that produce cholinesterase inhibition to which individuals are exposed on a daily basis such as solanine in potatoes, common colds and flu. In addition to natural sources, a significant portion of the population is being treated with pharmaceuticals that inhibit cholinesterase for conditions ranging from glaucoma to Parkinson's disease. These sources of inhibition have one thing in common; they produce measurable cholinesterase inhibition. This is important to consider with respect to the regulatory standard being considered in the case of carbaryl and its use in lawn care products.

The PBPK/PD modeling conducted by Connolly (2004) brings a degree of refinement to the "classical" pharmacokinetic approach, but frequently overestimates brain concentrations in rats as a function of time (e.g., Figure 15 peak brain level following oral dosing and Figure 30, peak brain level from all oral doses). Because the brain is the key target tissue for carbaryl AChE inhibition in rats and humans, the PBPK/PD model as currently parameterized appears to be conservative (tends to overestimate both concentration and effect in brain). One apparent reason for overestimation bias is the rapidity with which brain concentration rises in the current model, i.e., brain concentration of carbaryl is modeled to peak in ≤10 minutes, while actual measurements in rats demonstrate a peak level in 15-30 minutes following oral dosing. Further refinements to the PBPK/PD model will hopefully include more recently conducted cholinesterase monitoring data, as well as the cholinesterase monitoring done in conjunction with the data generated by Krolski et al. (2004a, 2004b). Future refinements of the PBPK/PD model might also include stochastic rather than deterministic representations of estimates for physiological and kinetic parameters.

Regardless of the method used (classical PK versus PBPK/PD) to analyze the new pharmacokinetic data in rats developed by Bayer CropScience, it is clear that the new data allow a more realistic basis for the estimation of the time course of internal dose in rats and humans. This information is critical because of the rapidity with which carbaryl is metabolized to cholinergically inactive molecules, and clearly peak brain concentration is more critical than average brain concentration. For a number of reasons, the MOEs estimated from these new data are conservative regardless of whether one uses classical PK or PBPK/PK.

1. The underlying basis for estimates of hand to mouth exposure are several-fold high based on inflated hand to mouth frequency, finger surface area contacting the mouth and degree of salvation.
2. Background was not subtracted in the biomonitoring study that served as the basis for total dosage.
3. Finally, the pharmacokinetic data demonstrate that rats and humans are very similar in terms of metabolism rate, types of metabolites, and dose response with

respect to cholinesterase inhibition requiring less than the 100-fold uncertainty factor historically used for inter- and intra-species differences.

The conclusion one can draw from use of the new pharmacokinetic data in refined exposure/dose and risk analyses is that when carbaryl is used according to the label, there is a reasonable certainty that no harm will result from post application reentry exposure.

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