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MEMORANDUM

SUBJECT: Formetanate HCl: Response to 5/21/09 Letter and Discussion of Toxicological Points of Departure and Resulting Risk Estimates.

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Attached is the Agency's response to Gowan's 5/21/09 letter and an evaluation of *in vitro* data included in that letter. This memorandum also reiterates deficiencies in the registrant–submitted comparative cholinesterase assay for formetanate HCl. A revised toxicological point of departure (PoD) is selected for formetanate HCl acute oral risk assessment based on the benchmark dose analysis of post-natal day 17 acetyl cholinesterase (rat). The basis for PoD selection is summarized here along with resulting dietary risk estimates.

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I. Summary

A recent re-evaluation of the acetyl cholinesterase (AChE) data of post-natal day 11 (PND11) and adult rats from the formetanate comparative cholinesterase (CCA) study (MRID 46618901) determined that the CCA study was unacceptable/non-guideline for both PND11 and adult rats (E. Reaves, 11/13/2008; TXR 0055042). Recently, as part of on-going research being conducted to support the cumulative risk assessment for the *N*-methyl carbamates, data for post-natal day 17 (PND17) rats (TXR 0055179) from the Office of Research and Development (ORD) have been made available. In lieu of data in PND11 pups, the PND17 data have been evaluated and considered acceptable for use in deriving a point of departure (PoD). A benchmark dose (BMD) analysis of the PND17 data has been conducted. The $BMDL_{10}$ of 0.006 mg/kg derived from the PND17 data have been incorporated into a newly revised dietary risk assessment. This assessment is presented below.

Key issues in the 'unacceptable' determination for the PND11 CCA study are the lack of dose response in RBC AChE data and significant number of 'DNRs' (i.e., sample which did not replicate) leading to re-analysis of samples and ultimately to possible underestimation of AChE activity. In response to the 11/13/08 CCA re-evaluation, the registrant (Gowan) submitted a letter on May 21, 2009. This letter contained *in vitro* data which suggest that the formetanate-enzyme complex was stable under conditions of the CCA study (Memo: May 21, 2009; Baker to Parsons) and Gowan's opinion that the CCA study provides an accurate measurement of AChE inhibition in both adult and PND11 rats. EPA has evaluated this *in vitro* study submitted by Gowan and has concluded that the *in vitro* study does not mimic the true conditions of the assay procedures in the CCA study and therefore is not appropriate as evidence of a stable enzyme-formetanate complex. The details of HED's response to the registrant's *in vitro* study are presented below.

Lastly, this memorandum provides estimates of dietary risk using the new points of departure derived from the PND17 data. These estimates include retention of the 10X FQPA Safety Factor for lack of PND11 data. The retention of the safety factor is based on available data for multiple N-methyl carbamates (NMCs) which show that PND11 pups are typically more sensitive than PND17 pups. This retention of the safety factor is consistent with the most current risk assessments for other NMCs without such acceptable data.

Using the new acute population adjusted dose (aPAD) (0.000006 mg/kg based on PoD= 0.006 mg/kg with 10X interspecies extrapolation factor, 10X intraspecies variability factor, and 10X FQPA factor), the acute dietary exposure estimates for food and water exceed HED's level of concern for the U.S. population and all reported population subgroups at the 99.9th percentile of exposure (>100% aPAD). Food and water were not combined since food only and water only risk estimates were each above the level of concern. Formetanate acute dietary exposure (food only) for the U.S. population was 3800% of the aPAD while the most highly exposed population subgroup (children 1-2 years old) was 8000% of the aPAD. Acute dietary exposure for water only for the U.S. population was 1900% of the aPAD while the most highly exposed population subgroup (all infants) was 7300% of the aPAD).

Food or drinking water risk estimates for formetanate would be substantially above the level of

concern even when the FQPA safety factor is reduced to 1X, i.e., if PND11 and PND17 rats are assumed to be equally sensitive. For the highest exposed populations, dietary exposures to formetanate would be 800% of the aPAD for children (food only) and 730% of the aPAD for infants (water only).

II. Evaluation of Available AChE Data from the CCA Study for Formetanate

The Agency acknowledges that the CCA study submitted by Gowan was one of the first studies performed for the NMCs and set the stage for evaluating sensitivity in juvenile rats for this class. Regrettably, the Charles River Laboratories (CRL) protocol was not optimized for the NMCs. This issue has been highlighted in the report by the FIFRA Science Advisory Panel (SAP) following the carbofuran SAP meeting in 2008. Of particular concern is the lack of dose response for RBC AChE inhibition which was shown in both carbofuran and formetanate studies. Some have suggested that washing of RBCs performed in the CRL protocol is the key issue with respect to the CRL protocol. Theoretically, the washing step could promote reactivation and which in turn leading to inability to accurately measure AChE activity in the inhibited sample. However, the Agency notes washing did not particularly impact carbaryl under conditions of the Nostrandt et al study (1993). As annotated in the Reaves 2008 memo, a substantial number of brain and RBC samples for the adult and juvenile pups (PND11) in the formetanate CCA study were re-analyzed. The time required to perform the re-analysis likely allowed reactivation of the enzyme thus likely underestimating AChE activity. The actual cause of the lack of dose-response data in RBC AChE is unknown. However, given that for some NMCs, RBC AChE data (a surrogate for lack of peripheral AChE data) have been shown to be more sensitive than brain, the lack of dose response data for RBC AChE is an important gap.

The source(s) of variability and measured AChE activity may never be definitively identified. Nevertheless, conditions in the laboratory appear to have impacted the dose-response compared with what would be expected, particularly at lower doses and for RBC AChE inhibition. For example, adult male RBC from the formetanate CCA study showed less inhibition than McDaniel et al (2007) who used a radiometric method. As indicated in Table 1 below, the formetanate CCA study showed 3-fold less RBC inhibition than in the McDaniel study at similar doses of 0.6 mg/kg and 1.5 mg/kg doses. Similarly, the formetanate CCA study showed about 3-fold less inhibition in brain AChE inhibition at the lowest dose compared with the McDaniel et al (2007) study (Table 2). At higher doses, the amount of RBC and brain AChE inhibition are similar between the McDaniel et al (2007) and the formetanate CCA study in adult rats.

Table 1. Comparison of RBC AChE inhibition in adult male rats

	% RBC AChE inhibition in adult male rats	
Dose	**McDaniel et al. 2007 study**	**Formetanate CCA study (MRID 46618901)**
0.6 mg/kg	20%	7%
1.5 mg/kg	40%	13%
Highest dose	50% (5.0 mg/kg)	29% (3.0 mg/kg)

Table 2. Comparison of brain AChE inhibition in adult male rats

	% brain AChE inhibition in adult male rats		
Dose	**McDaniel et al. 2007 study**	**Formetanate CCA study (MRID 46618901)**	**Formetanate ACN study (45314201)**
0.6 mg/kg	40%	16%	
1.5 mg/kg	50%	39%	34-45% (1.0 mg/kg)
3.0 mg/kg	60%	50%	

III. Revised Point of Departure

As part of on-going research to support the cumulative risk assessment for the NMCs, ORD has performed dose-response and motor activity on PND17 pups for 7 individual NMCs. These experiments were conducted to determine information needed to plan and conduct a mixture study with these 7 NMCs. The PND17 data from this mixture study the individual chemical dose response studies have been evaluated by HED. Given the robust dose response and use of the radiometric method in the AChE assays, the formetanate PND17 data were determined to be appropriate for use in risk assessment. The PND17 study report will be provided to Gowan when it becomes available.

Generally, for NMCs, juvenile rats have been shown to be more sensitive than adult rats. Similar to the formetanate risk assessment which supported the RED, data from juvenile rats have been used in the most recent risk assessments for several NMCs. As such, the PND17 study currently provides the most robust data in juveniles for formetanate (Table 3).

The PND17 BMD analysis and selection of acute oral PoD were summarized in a 5/18/09 memo (J. Liccione, TXR 0055179, D364562).

As shown in Table 3 below, the brain BMD_{10} and $BMDL_{10}$s for the ORD PND17 data are 5-6 fold lower than those from Gowan's CCA study. Moreover, the RBC AChE BMD_{10} and $BMDL_{10}$ are lower than those for brain in the ORD data. This pattern also is consistent with carbofuran and methomyl. HED has determined that the $BMDL_{10}$ of 0.006 mg/kg will be used for risk assessment.

Table 3. AChE BMD_{10} and $BMDL_{10}$s for PND17 and PND11 male pups for formetanate

Age	Brain AChE	RBC AChE
PND17 Male (ORD)	BMD= 0.0309 BMDL=0.0188	BMD=0.0134 BMDL=0.0060

PND11 Male (Gowan CCA study*)	BMD=0.188 BMDL=0.098	No dose-response

*PND11 male BMD and BMDL estimates based on the analysis by Setzer, 5/25/2006 as part of the NMC CRA.

With respect to the 10x FQPA factor, the Agency is retaining the full 10x in the absence of a high quality PND11 study with good dose-response (range of doses) and quality RBC and brain AChE data. The sensitivity of the PND11 pups to adult rats has been consistent for carbofuran, carbaryl, methomyl, and oxamyl. Furthermore, PND11 pups have been shown to be more sensitive than PND17 pups for carbofuran and carbaryl. This approach of retaining the 10x is also consistent with the most recent risk assessments for aldicarb and carbofuran.

The PoD for formetanate acute dietary risk assessment is 0.006 mg/kg ($BMDL_{10}$). The new acute PAD is 0.000006 mg/kg/day (based on PoD= 0.006 mg/kg with 10X interspecies extrapolation factor, 10X intraspecies variability factor, and 10X FQPA factor).

IV. HED Response to the Gowan In Vitro Study submitted May 21, 2009

A. Formetanate-enzyme complex was stable under conditions of the study

Gowan submitted preliminary *in vitro* work that was performed by Charles River Laboratories (CRL) in March, 2005. This *in vitro* work was done to "determine the stability of the enzyme-formetanate complex *in vitro* over 24 hours, at physiologically temperature (37°C) and also at 26°C which was the assay temperature specified by the Agency to mitigate potential reactivation of the AChE." The Agency has evaluated the submitted *in vitro* data and has concluded that this study does not provide evidence of a stable enzyme-formetanate complex under conditions of the CCA study. Specifically, the *in vitro* study took the enzyme and added formetanate to the solution at fixed concentrations. This created an immediate (within seconds) equilibrium situation where both sides of the concentration equilibrium equation did not change and the rate of carbamylation vs. decarbamylation was the same. Therefore, the complex would be expected to be stable for 6 hours, and the "recovery" was likely breakdown of the chemical or enzyme. Furthermore, the ex vivo situation where the enzyme:inhibitor complex (inhibited tissue) is diluted forces the reaction to the left, the rate of which depends on the affinity to the enzyme. It is known that carbamates dissociate from the inhibitor quickly and hence the basis of concern for diluting carbamylated tissues. At some point in time the reaction will reach equilibrium in the diluted solution, at which point no further change over time will occur. However, it would no longer provide an accurate measure of how much of the enzyme was inhibited.

Therefore, the 2 assay scenarios are not the same and do not provide evidence of a stable enzyme-formetanate complex for any length of time. An appropriate evaluation of complex stability would be to mix the enzyme and formetanate, dilute according to assay procedures in the CCA study, and then sample periodically. As with other *N*-methyl carbamates, the reactivation of the enzyme with formetanate is expected to occur within minutes and therefore the time at which the sample is analyzed is one critical step to accurately measuring inhibition of the enzyme.

HED does acknowledge that based on time course data reported in Padilla et al (2007) study in which adult rats were analyzed with the radiometric assay, the adult half-life for formetanate was

approximately 4 hours for the brain. This 4 hour half-life is longer than most of the NMCs which is typically 1 to 2 hours (2007 NMC CRA) and may suggest somewhat different pharmacokinetics for formetanate. However, at this time, data are too limited to make any determination with respect to how formetanate could interact differently with AChE enzyme compared with other NMCs.

B. Assays performed within the stability window

Gowan suggests that the assay time of 60 minutes or more that occurred in the CCA study was adequate based on the stability of the formetanate-enzyme complex for 6 hours. However, as described above, EPA does not agree with Gowan about the conclusions with respect to the *in vitro* data provided in the May, 2009 memo.

C. Formetanate hydrochloride is not carbofuran

The Agency agrees that the potency and time-course of formetanate and carbofuran are not exactly the same but does note that they share the same mechanism of action and thus share many of the same toxicological properties. The *in vitro* study provided by Gowan does not provide evidence of stability for the conditions performed in the CCA study. Ultimately, the Agency is relying on the PND17 data regardless of the conditions performed in the CCA study.

D. Additional considerations

Gowan indicates that the cholinesterase assay methodology employed in the CRL relative sensitivity study was mandated by the Agency itself. Although Gowan cited references (Ellman 1961, Hunter 1997) pertaining to modifications in cholinesterase assay methodology for a meeting with HED in 2004, few details concerning the protocol were provide to HED. As stated in the Doherty memo (1/5/2005, TXR0052987), "few details for blood and brain sampling, preparation for assay and techniques that will be used to minimize reversal of inhibited enzyme was described". The memo further states that "providing the citations for the Hunter and Ellmans papers is not sufficient." The conclusion of the memo states that "HED has commented on the protocol as submitted it is the Registrant's responsibility to provide a study that meets acceptable standards for quality regarding housekeeping aspects, quality data with acceptable variance and report preparation."

V. Summary of Dietary Risk Estimates

A. Acute Dietary Risk Estimates using Revised Point of Departure

An acute dietary assessment for exposure to formetanate through food and water was performed using the revised PoD (BMDL=0.0060, PND17 RBC) and an aPAD of 0.000006 mg/kg/day (W. Donovan, D364563, 5/12/09). For food, this assessment incorporated updated percent crop treated information and Pesticide Data Program (PDP) results from 2007 for nectarines and peaches. All other residue assumptions were the same as those used in the previous analysis (S. Stanton *et. al.*, D328181, 5/17/06). For water, the Environmental Fate and Effects Division provided surface water modeling EDWCs based on formetanate application to onion, citrus, apples, pears, peaches, and alfalfa (I. Abdel-Saheb, D359092, 2/4/09). A screening level dietary assessment was performed using the EDWC from citrus (CA) as a point estimate. The citrus EDWC represents a lower bound estimate compared to other crop scenarios, which resulted in higher EDWCs than citrus.

The acute dietary exposure estimates for food and water exceed HED's level of concern for the U.S. population and all reported population subgroups at the 99.9th percentile of exposure (>100% aPAD). Food and water were not combined since food only and water only risk estimates were each above the level of concern. Formetanate acute dietary exposure (food only) was estimated at 0.000228 mg/kg/day for the U.S. population (3800% of the aPAD) and 0.000482 mg/kg/day (8000% of the aPAD) for the most highly exposed population subgroup (Children 1-2 years old) (results Table 4 below).

The resulting acute dietary exposure estimates for water only exceed HED's level of concern for the U.S. population and all reported population subgroups at the 95th percentile of exposure. Formetanate acute dietary exposure (water only was estimated at 0.000115 mg/kg/day for the U.S. population (1900% of the aPAD) and 0.000435 mg/kg/day (7300% of the aPAD) for the most highly exposed population subgroup (All infants) (results Table 4 below).

Most of the estimated food only acute exposure was determined to result from consumption of apples, oranges, and pears (particularly apples, and to a lesser extent, pears). When the top three contributing food items (apples, oranges, and pears) are removed from the analysis, all population subgroups have exposure levels below 1000% aPAD (children 1-2 yrs at 810% aPAD), but are still of concern to HED.

A food only sensitivity analysis was conducted by substituting zeros in place of ½ LOD (method level of detection) values to examine the impact of assuming ½ LOD for treated commodities with no detectable residues. This substitution resulted in somewhat lower risk estimates than what was obtained using ½ LOD values (e.g., for children 1-2 yrs: 8000%aPAD using ½ LOD vs. 6500%aPAD using zeros) but does not result in acceptable risk estimates for the food uses of formetanate.

The dietary assessment does not include the recently submitted onion field trial data (not yet reviewed) which resulted in measurable residues. This assessment, like the 2006 assessment, assumed no detectable residues in onions.

Table 4. Results of Acute Dietary Exposure Analysis

Population Subgroup	aPAD (mg/kg/day)	FOOD ONLY 99.9th Percentile		WATER ONLY 95th Percentile	
		Exposure (mg/kg/day)	% aPAD	Exposure (mg/kg/day)	% aPAD
General U.S. Population	0.000006	0.000228	3800	0.000115	1900
All Infants (< 1 year old)		0.000478	8000	**0.000435**	**7300**
Children 1-2 years old		**0.000482**	**8000**	0.000181	3000
Children 3-5 years old		0.000406	6800	0.000165	2800

Population Subgroup	aPAD (mg/kg/day)	FOOD ONLY 99.9th Percentile		WATER ONLY 95th Percentile	
		Exposure (mg/kg/day)	% aPAD	Exposure (mg/kg/day)	% aPAD
Children 6-12 years old		0.000239	4000	0.000115	1900
Youth 13-19 years old		0.000151	2500	0.000094	1600
Adults 20-49 years old		0.000117	2000	0.000107	1800
Females 13-49 years old		0.000120	2000	0.000097	1600
Adults 50+ years old		0.000126	2100	0.000108	1800

B. Comparison of Dietary Risk Estimates using Different FQPA Safety Factors

Table 5 compares the new dietary risk estimates (highest exposed population, infants and children) for food and water using the new PoD. The second column illustrates the risk estimates that would result if the 10X safety factor were reduced to 1X, i.e. if PND11 and PND17 rats are assumed to be equally sensitive. Food or drinking water risk estimates for formetanate would be substantially above the level of concern even when the FQPA safety factor is reduced to 1X.

Table 5. Comparison of %aPADs for formetanate

	New Risk Assessment Using Current Policy & Practice	If 10X were reduced to 1X (PND17 and PND11 assumed to be equally sensitive)
aPAD (mg/kg/day)[1]	0.000006	0.00006
%aPAD food only (Children 1-2 yrs; 99.9th percentile)	8000.	800.
%aPAD water only (Infants; 95th percentile)	7300.	730.

VI. Occupational and Residential Exposure Assessments

There are no residential uses of formetanate HCl so a residential assessment is not warranted. Occupational handler and post-application exposures are possible. The Agency is currently re-evaluating the PoDs previously used for occupational workers. A benchmark dose analysis on the available data may be appropriate for selecting new PoDs. A revised occupational assessment would be needed using the new PoDs.

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