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REPORT:
FIFRA Scientific Advisory Panel Meeting, December 8, 1998, held at the Sheraton Crystal Hotel, Arlington, VA

II - A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

A Retrospective Analysis of Developmental Neurotoxicity Studies

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Larry C. Dorsey
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date:_____

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Dr. Ernest E. McConnell
Chair
FIFRA/Scientific Advisory Panel
Date:_____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT SCIENTIFIC ADVISORY PANEL MEETING

II - A Retrospective Analysis of Developmental Neurotoxicity Studies

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding a retrospective analysis of developmental neurotoxicity studies. Advance public notice of the meeting was published in the *Federal Register* on November 13, 1998. The review was conducted in an open Panel meeting held in Arlington, VA, on December 8, 1998. The meeting was chaired by **Dr. Ernest E. McConnell** of Toxpath, Inc. **Mr. Larry Dorsey,** SAP Executive Secretary, served as the Designated Federal Official.

Participants

FIFRA Scientific Advisory Panel Members:
Dr. Ernest E. McConnell, Toxpath, Inc., Raleigh, NC
Dr. Ronald J. Kendall, Director and Professor, The Institute of Environmental and Human Health/ Texas Tech University Health Sciences Center, Lubbock, TX
Dr. Fumio Matsumura, Professor, Institute of Toxicology and Environmental Health, University of California at Davis, Davis, CA
Herb Needleman, M.D., Professor of Psychiatry and Pediatrics, School of Medicine, University of Pittsburgh, Pittsburgh, PA
Dr. Mary Anna Thrall, Professor, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, CO

FQPA Science Review Board Members:
Cynthia Bearer, M.D. Ph.D, Assistant Professor, School of Medicine, Case Western Reserve University, Cleveland, OH
Dr. Janice Chambers, Professor, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS
Dr. Robert Chapin, National Institute of Environmental Health Sciences, Research Triangle Park, NC
Dr. Luz Claudio, Professor, Division of Environmental and Occupational Medicine, Mount Sinai Medical Center, New York, NY

George Lambert, M.D., Associate Professor of Pediatrics, Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ
Dr. Charles Mactutus, Professor, Tobacco and Health Research Institute, University of Kentucky, Lexington, KY
John O' Donoghue, V.M.D, Ph.D., Director, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY
Dr. William Slikker, Director, Division of Neurotoxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AK
Dr. Thomas J. Sobotka, Food and Drug Administration, Laurel, MD
Dr. John Wargo, Professor, School of Forestry and Environmental Studies, Yale University, New Haven, CT

Oral statements were received from the following individuals:
Dr. Abby A. Li and Dr. Larry B. Sheets, American Industrial Health Council and American Crop Protection Association
David Wallinga, M.D., Natural Resources Defense Council.
Dr. Michael W. Gill, DEET Joint Venture/Chemical Specialities Manufacturers Association

Written statements were received from:
American Crop Protection Association
The Dow Chemical Company
Learning Disabilities Association of America
Natural Resources Defense Council

Summary of Agency Presentations

Susan Makris (EPA, Office of Pesticide Programs) described the history and content of the standard developmental neurotoxicity testing guideline (OPPTS 870.6300) and summarized the study methods and results (maternal and offspring) of all developmental neurotoxicity studies received and reviewed within OPPTS as of November, 1998. This data set included nine studies on pesticides and three on toxic substances (solvents). Ms. Makris also presented summaries of the data on prenatal developmental toxicity, reproductive toxicity, and acute and subchronic adult neurotoxicity for the same chemicals; the studies, NOAELs and endpoints selected for acute and chronic dietary risk assessment for these chemicals were included as well. The offspring NOAELs from the developmental neurotoxicity studies were compared with those of the other studies within each chemical database, as well as with those studies that were selected for risk assessment. Specific issues that were raised during the process of reviewing the data were addressed in some depth and are expected to provide the focus for future discussions on guideline revision.

Panel Response to Agency Presentation and Questions

Revision of Developmental Neurotoxicity Guidelines

The Panel agreed with the Agency that the developmental neurotoxicity guidelines should be revised based on reviews of submitted data. This will assure that the Agency will maintain current in the understanding of brain development and function, and the effects of chemicals thereon. The Agency should take into consideration the frequency and manner in which such revisions should be pursued. The Panel recognized the huge amount of effort and resources that this demands, and commends the Agency for investing these resources. Developmental neurotoxicity testing (DNT) must be further refined to develop more sensitive endpoints which are relevant to significant outcomes in humans such as learning disabilities and behavioral issues.

Flexibility in Conduct of Studies

The Agency should be acknowledged for their willingness in allowing flexibility to pesticide registrants in the conduct of DNT studies concerning such issues as which morphology testing (and other specific tests) will be used for functional effects and consideration of new methods. Such flexibility helps ensure the best quality data of the kind most appropriate for the individual compound(s) under study. Flexibility would more easily allow considerations of characteristics of individual compounds which impact specific toxicity profiles specific for each compound.

Agency Data Evaluation Reviews

There is very marked variability in the Data Evaluation Review, some as short as two pages. It is difficult to ascertain what was done and what was found. As an example, the documents did not clearly state that appropriate analysis with "litters" had been done.

Learning/memory tests

The majority of the Panel agreed that learning/memory endpoint should be required, even though the submitted learning/memory tests may not be the most sensitive indicator of learning/memory function. In submitted studies where learning/memory tests were practiced, this typically entailed a water maze task. Several employed more apical type tasks, e.g., spatial delayed alteration and delayed matching to position. Unfortunately, it was not clear that any of the tests were used with much sophistication. The interpretation of tests requiring food deprivation to establish motivation for task performance must take into consideration the possibility that the disposition of the compound may be altered by reduction of fat stores. However, one Panel members disagreed, concluding that learning memory tests are the most sensitive indicator of learning/memory function. In addition, no data were submitted that could be adequately used to compare learning/memory tests for sensitivity.

Agency Questions

The Agency presented the following questions to the SAP regarding a retrospective analysis of developmental neurotoxicity studies. The questions are keyed to the draft background document entitled *A Retrospective Analysis of Twelve Developmental Neurotoxicity Studies Submitted to the USEPA Office of Prevention, Pesticides, and Toxic Substances, November 12, 1998.*

1) Does the Panel agree with the conclusions, that based upon these data:

A) That the data show a variety of effects on functional development of behavior and the structural development of the nervous system, as currently assessed by the methods in the developmental neurotoxicity guideline, and that these methods constitute a reasonable set of general methods for assessment of toxicity to the offspring?

While the Panel agreed that the data adequately demonstrate effects on functional development of behavior and structural development of the central nervous system, the Panel was divided whether the methods identified are reasonable for assessment of toxicity to the offspring. The current review of twelve DNT studies provides additional information to assess the utility of the Agency DNT protocol, but does not suggest that the protocol revised in August, 1998 should be altered at this time. The tests of the 12 chemicals reveal that the DNT guideline included sensitive measures of maternal toxicity, developmental toxicity, and developmental neurotoxicity over a range of dose levels. The NOELs resulting from the DNT studies were in the range of the NOELs identified in other sensitive assays for such endpoints as chronic toxicity, carcinogenicity, and neurotoxicity.

However, other Panel members did not concur. The reasons for disagreement were:

- Endpoints were not sensitive enough to detect neurotoxicity associated with significant endpoints in humans.
- Exposure of rat fetus/pup were not shown to be equivalent to human fetus/infant during equivalent stages of brain development, both with respect to third trimester equivalent exposure (lactional exposure in rat/transplacental in human), and in length of total exposure (does not cover extensive postnatal period of brain development in humans).
- No data were submitted for chemical/metabolite tissue level in rat fetuses/pups to demonstrate adequate exposure.

B) That effects on neurological development, as currently assessed in the DNT, can be a sensitive indicator of toxicity to offspring in comparison to effects found in either developmental or reproduction studies, or in comparison to adult neurotoxicity studies?

The majority of the Panel disagreed with this statement and strongly expressed common concerns as to why the DNT is not more sensitive than either developmental or reproductive studies. Their common concerns were:

- The DNT is not more sensitive in its current form, which, given what is known in the broader neuroscience and pediatric community, is in contrast to the fact that developmental neurotoxicity occurs in the absence of maternal toxicity. Therefore, the current form of the DNT guidelines, or how developmental neurotoxicity testing was conducted in the 12 studies, is not a sensitive indicator of toxicity to the offpspring. This identifies a weakness with the DNT guidelines and/or the 12 submitted studies.

- Even if the DNT is not more sensitive than other types of testing, it provides important qualitative data not obtained in other types of testing. Thus, testing DNT should be required to determine the presence or absence of developmental neurotoxic effects.

- The small number of studies (12), many lacking compliance to the DNT guideline, is inadequate to provide support of DNT as a sensitive indicator of toxicity to offsprings.

- The chemicals represented in the 12 studies do not represent the full spectrum of chemicals in use. Therefore, the Agency's statement can not be made for all chemicals.

- Future reviews should provide the statistical power associated with the endpoints used in the DNT so that the size of the biological change required to reach statistical significance is known. This would provide important information as to the innate sensitivity of the test.

Other panel members did agree with the Agency that the DNT can be a sensitive indicator of toxicity to offspring. In the current series of 12 studies, the DNT showed statistically positive results at dose levels that were in line with the lowest observed dose levels for systemic toxicity, developmental toxicity, and carcinogenicity bioassays. This series of DNT studies provides the only dataset where endpoints for DNT, systemic toxicity, and developmental toxicity have been collected using the same protocols with concurrent data collection for each of the endpoints of concern. Requests by the SAP for an analysis of available DNT studies were made of the Agency specifically because these types of comparative data were not available prior to the Agency's analysis of the 12 studies. Of the 12 studies reviewed, two (or 16%) of the studies had DNT endpoints which were more sensitive than either systemic or developmental toxicity endpoints. In particular, the DNT results for emamectin demonstrate that DNT is sensitive to neurobehavioral effects in offspring when there are no neuropathological effects in the offspring and no evidence of maternal systemic toxicity or neurotoxicity.

Table 9 of the Agency's background document is instructive. For the nine chemicals for which DNT data and other endpoint data are available, only one DNT pup endpoint (decreased auditory startle in pups given molinate) was chosen for pesticide risk assessment and this endpoint was not the most sensitive endpoint. For the molinate risk assessment, peripheral neuropathy in

adult animals provided the lowest NOEL for risk assessment.

While the Agency's background document compares data for the DNT studies with other study endpoints (Table 10 of the Agency's background document where DNT results appear to occur at lower dose levels than developmental toxicity endpoints) to demonstrate the sensitivity of DNT endpoints, this type of comparison can lead to misinterpretation because the studies were not conducted concurrently and thus variability in the results between studies can mask true effects. One Panel member provided a comparison of the Agency data which could have been performed to eliminate variability due to dispersion of data collection over time. This analysis is presented in Appendix 2.

Each DNT study collects not only neurotoxicology data on the pup, but also collects systemic toxicity and neurotoxicity data on the dams, and developmental toxicity data on the pups. The data for these four types of endpoints have been extracted from the Agency reviews of each study and summarized along with the respective NOELs and LOELs, and the endpoints which supported the LOELs. When broken out in this manner, the data show that while the DNT was sensitive to many different types of neurotoxicity endpoints occurring in the pups, only two chemicals, molinate and emamectin, had endpoints for developmental neurotoxicity which occurred at dose levels that were less than the NOELs for maternal or developmental toxicity.

Appendix 3 through 5 present decision matrixes which show the distribution of the NOELs for the 12 studies with regard to relative sensitivity between developmental neurotoxicity and maternal toxicity (Appendix 3), developmental neurotoxicity and developmental toxicity (Appendix 4), and developmental neurotoxicity and maternal or developmental toxicity (Appendix 5). In each of these figures, lower NOELs for developmental neurotoxicity are indicated on the vertical axis by the zone marked "yes". On the horizontal axis, the presence or absence of maternal toxicity or developmental toxicity are indicated by the zones marked "yes" or "no". Appendix 5 demonstrates that all but two of the chemicals show maternal toxicity or developmental toxicity at levels that are at or below the NOEL for developmental neurotoxicity. Whether the molinate result is a true result is somewhat speculative as the data supporting the lowest effect level for developmental neurotoxicity is not a robust indicator of neurotoxicity. The decision on picking the LOEL for the molinate DNT study was a significant decrease in startle amplitude at all dose levels on day 23 post partum. This effect was the only effect observed at the low dose level, and it occurred in only one sex at one time point at this dose level. A subsequent assay on day 61 showed no effect at this dose level.

C) That effects on brain weight, histopathology, and morphometric assessments also can be sensitive indicators of toxicity to the offspring?

Panel members were divided with this statement. Those concurring with this position commented that poor maternal nutrition as a manifestation of maternal toxicity leading to lower brain weight is an important aspect of DNT. Developmental neurotoxicity is the endpoint, whether it is a direct or an indirect result of the exposure. If a pesticide causes reduced body size

and reduced brain weight, this latter is a neurotoxic effect, regardless of the causal path from pesticide to brain. While whole brain weight, histopathology and morphometric assessments may not be more sensitive indicators of toxicity, they are valuable indices and should continue to be collected.

Other positions were also expressed by Panel members. The Agency has stated its policy with regard to pup brain weight as, "A change in brain weight is considered a biologically significant effect. This is true regardless of changes in body weight, because brain weight is generally protected during under nutrition or weight loss, unlike many other organs or tissues. It is inappropriate to express brain weight changes as a ratio of body weight and thereby dismiss changes in absolute brain weight". The Agency's background document cites Allen (1995) as supporting the Agency's policy decision. The Allen (1995) citation is a positive control study for the molinate DNT study which was sent to the SAP for review. The conclusion of the Agency reviewer was that the findings for this study included " (a) a shortening of the time to vaginal opening in the female offspring of the dams on both the 80% restriction and the meal-fed dietary regimens and (b) decreased brain weight in the female offspring of the meal-fed dams at day 63 postpartum." Thus, it is clear from this study that simple under nutrition can induce both developmental delays and reduction in brain weight without chemical exposure (as presented in Appendix 2 of this report). Therefore, some Panel members concluded that it would seem appropriate for the Agency's policy to recognize that altered nutrition induced by chemical exposure can lead to reduced brain weight and that while this effect may be biologically significant, it may not be evidence of chemical-induced neurotoxicity in the pup. In addition, given the caveat that chemical-induced under nutrition may result in decreased pup brain weight, a significant, dose-related decrease in brain weight may be a sensitive indicator of neurotoxicity.

Neuropathology has long been one of the fundamental endpoints for neurotoxicity detection and risk assessment and is generally regarded as a sensitive endpoint for detection of neurotoxicity. It is not clear that the data presented in the current study either reinforce or diminish this opinion.

There are a number of papers in the literature that indicate that morphometrics can provide quantification of morphological changes. The Agency, in its review of the DNT studies, notes the importance of allowing professional judgment to be used by the pathologist in performing the morphometric analysis. In measuring morphometric endpoints, the pathologist provides an important quality control function by determining the adequacy of the sample for morphometry and whether confounding factors are present which would alter morphometric findings. It is appropriate for the pathologist to consider the statistical significance of morphometric endpoints based on the slide review. Yet for two studies (chlorpyrifos and carbaryl), the Agency reviewers dismissed the pathologists' conclusions that treatment related effects were not present without conducting a slide review. These decisions by the Agency reviewers were based on conclusions about the statistical significance of morphometric endpoints as toxicologically significant, even when the morphology and distribution of morphometric changes would indicate that the results were not significant. If the original pathologist's opinion needs to be reviewed, it is recommended

that the Agency include a slide review in its process. Peer review processes used by other government agencies, such as the National Toxicology Program, could be used as models for the Agency's review process.

Regional brain weights are sensitive indicators of neurotoxicity and could be used to help guide further analyses including morphometry and histology. Changes associated with lower doses of neurotoxicants may be observed using ultrastructural microscopic anatomy studies and quantitative counts of dendritic complexity using rapid Golgi stains. Histopathological evaluation is a necessary and integral component of DNT. Morphometric analysis appears to have potential utility but the methodology needs to be standardized. Morphometric analysis can be improved by computer-aided image analysis. These methods should be explored to enhance the current guidelines.

D) That these functional and morphological effects both seem to occur with similar frequency, i.e., appear to be correlated?

While the Panel agreed that functional and morphological effects occur with similar frequency and at similar dose levels, they disagreed that they were correlated. Correlation requires statistical analysis, coincident is a better term. Even though frequencies were similar, dose levels were not. Similar frequencies do not imply correlation, similar frequencies can occur with zero correlation. Neither measure used here is sensitive enough. Behavioral effects are expected to be more frequent than morphologic effects if proper endpoints are selected. Scientists cannot rely on data from one endpoint without data from other endpoints and both are necessary components of a comprehensive neurotoxicity evaluation. In addition, in none of the Agency reviews of the DNT data was there an attempt to assemble all of the neurotoxicology data into a coherent descriptive diagnosis of the neurotoxicity observed.

E) That neurobehavioral effects in dams as assessed by detailed clinical observations in the DNT can be sensitive indicators of toxicity in adults.

This would not be considered as critically as a more objective measure. Due to the limited size of the dataset, it is not clear if maternal detailed clinical observations are a sensitive indicator. In assessing the occurrence of neurotoxicity in dams, all of the available data for adult animals should be assessed and integrated to reach a final conclusion about neurotoxicity. Systemic clinical observation needs to be more precisely defined. The neurobehavioral assessment in dams from DNT studies may not be as complete as the assessment occurring as part of an adult neurotoxicity study. However, neurobehavioral effects observed in the dams included in the DNT studies did appear to be sensitive indicators of toxicity in adult animals.

Concerning maternal toxicity, it should be considered an important endpoint as it can have important implications for the fetus/newborn. The Panel was divided whether it was a sensitive indicator of adult toxicity. One problem concerned the limited exposure times and the low doses used in DNT. In addition, the Panel did not conclude that maternal toxicity should be a substitute

for neurotoxic tests in adult (non-pregnant) animals.

2) Does the Panel have comments on any of the specific issues raised in the Agency's background paper?

A) Route of administration

The Agency's current approach seems a reasonable balance between conflicting priorities. These conflicting priorities include: (a) the need to have data collected by exposure routes relevant to human exposure potential, (b) providing maximum treatment exposure, (c) assuring treatment is inside the test animal by one route of administration, (d) the need to limit disruption of dam-pup interactions by the presence of bandaging used in dermal studies, (e) separation of the dam and pup which occurs with inhalation exposures, (f) need for accurate exposure information for completion of risk assessments, and (d) consistency with previous studies.

While inhalation and dermal exposure routes may be more relevant than oral gavage or dietary administration under some circumstances, the logistics involved in conducting DNT studies by these routes is significant and disruption of dam-pup interactions may become a significant confounder for interpreting DNT studies. Oral gavage of the dam provides a more easily calculated dose level. However, the Panel recognizes that direct gavaging of pups is a very stressful procedure, which could confound results because of the trauma induced in the pups. Thus, dietary administration is more likely to provide the least opportunity to disturb dam-pup interactions. In addition, the use of appropriate controls should enable, in most instances, the DNT studies to be validly interpreted. Obviously, there should be some consideration for reasonableness in study design. However, the usefulness of the DNT studies for risk assessment could be notably reduced if selection of route of administration was routinely based solely on convenience and not on relevance to human exposure and to information developed in previous studies.

The Panel also commented on direct dosing of pups. While several Panel members agreed that direct dosing was acceptable, if the objective of DNT is to provide a better model for assessing treatment related effects during the postnatal period of nervous system development, it would be more appropriate to consider a separate experiment protocol guideline in which the offspring would be dosed directly during the postnatal period of development.

B) Duration of treatment

The series of studies included in the Agency's review has demonstrated that dosing up through lactation day ten, when the nervous system is undergoing significant development, resulted in both functional and morphological changes in the nervous system. The DNT study protocol has been designed to look for effects resulting from exposure during gestation and early lactation (essentially toxicity occurring through exposure to the mother). If the scope of the testing paradigm is expanded to include a longer period of development, such as through PD21, it is

appropriate to adjust the dosing period accordingly. What will be lost in expanding the scope of the study will be the ability to differentiate effects mediated through exposure to the dam versus those mediated through exposure to the pup. Duration of treatment beyond lactational period may be problematic. It is best to direct exposure to pups through brain maturation to model the extensive postnatal growth and development of the human brain. However, the Panel acknowledges that this is labor intensive. Dams should be exposed prior to pregnancy and throughout pregnancy to model increased body burdens as a contributing source of exposure to the fetus.

Toxicokinetics needs to be considered in terms of the duration of treatment. As an example, organophosphate toxicity involves the inhibition of cholinesterases. However, it should be noted that the majority of organophosphorus insecticides (approximately 75%) are rather poor inhibitors of AChE *per se.* The parent inhibitors must be metabolically activated to become AChE inhibitors. Such reactions occur within the microsomal mixed function oxidase systems located principally in the liver. Common examples of this process are parathion to paraoxon, malathion to malaoxon, and chlorpyrifos to chlorpyrifos oxon. One Panel member indicated that in one of the Agency Data Evaluation Reviews, the nursing pup may not show such conversion, and hence will not be exposed. Continuing dosing beyond birth into the neonatal period would thus be pointless. However, another Panel member responded that neonates do possess bioactivation potential and therefore can be exposed from lactational exposure to the parent insecticide. Other insecticides do not require bioactivation. Also, it is possible that the oxon could be transferred in milk, although this is less likely than with the parent insecticides. It is critical that both the activation and detoxication potential be considered.

C) The use of combined protocols

The Panel agrees that protocols should be combined, combining the 2-generation reproduction and the developmental neurotoxicity studies is desirable for internal relatedness. Thus, all studies on one compound should use the same dose (if possible), same batch of compound, and same strain and species of test animal. Consistency of these considerations leads to the strength of the dataset and subsequent data analysis. Two of the studies (DEET and Chemical X) included in the Agency's background document provide evidence that combined protocols can be successful. Where the DNT test is triggered, it may make sense to suggest combining the DNT test with other tests. A significant concern in combining the tests is the logistical capabilities of the laboratories conducting the studies. The DNT test, as well as the other tests for developmental toxicity, are large studies which require excellent logistical support in addition to significant technical and scientific expertise. In addition, combining protocols should not be at the expense of compromising the data of either of the combined protocols.

If protocols are combined, several issues need to be considered. First, the prenatal study is used as a trigger for requiring the DNT study. How will this change if protocols are combined? Second, are the criteria for dose selection for both studies compatible?

Several Panel members disagreed with the public comments by the American Industrial Health Council (AIHC) for combining the 2-generation reproduction and developmental neurotoxicity studies. First, AIHC's proposal did not address longitudinal assessments - a hallmark of DNT testing. Second, there was a lack of adequate behavioral assessment approaches or fixation by perfusion.

D) Biochemical measures, including cholinesterase inhibition

The Panel concluded that a recommendation to measure cholinesterase (when testing a compound expected to result in cholinesterase inhibition) or other biochemical targets (when these are known) in dams and neonates should be included in future updates of the DNT test guideline. While the Panel acknowledges that identification of such compounds may not be a measure of neurotoxicity (only a measure of exposure), it may be relevant in assuring the dam has received an appropriate dose of the test compound. It should be emphasized that a level of cholinesterase inhibition which results in adverse effects has not been clearly understood.

One of the major weaknesses of the current design is that the guidelines do not mandate that SOME explicit measure of fetal and pup exposure be made. While limited conclusions could be made in the absence of such information, such pharmacokinetic data is necessary to adequately characterize neurotoxic effects of the test compound during pregnancy. However, merely knowing that some component of the administered test compound entered the fetus/pup does not necessarily provide evidence that the neurotoxic metabolite entered the fetus/pup.

E) Pharmacokinetic data

Pharmacokinetic data are an essential component of risk assessment. The Panel questioned whether such data should be required in the DNT protocol since collecting such data may be too intrusive in the DNT study. It is not clear from the Agency's background document whether pharmacokinetic data were available from other FIFRA required studies that could have been extrapolated for use in the developmental toxicity studies. The Agency has not made it clear what pharmacokinetic data it is interested in receiving, thus it is difficult to respond to the Agency's question in a meaningful way. Simple pharmacokinetic data may provide useful information for assessment of biologic effects. Thus, the Agency should consider the utility of requiring and utilizing such data.

F) Simple Morphometric Analysis

While morphometric analyses are worthwhile for further conformation of developmental neurotoxicity testing, it is not a sensitive assay. The utility of such data is that they allow the identification of subtle changes to the structure of the brain. It is prudent to consider that any changes in the structure of the brain are considered adverse unless shown otherwise. Thus, until a more extensive database is developed about the appropriate approaches for measurement and selection of regions to measure are developed, morphometric analysis should not be required.

It appears from the reviews of two studies submitted in the Agency supporting documents (i.e., carbaryl and chlorpyrifos) that the morphometric data that were collected were not properly integrated into the overall pathology examination by the Agency reviewers. In these two studies, the diagnoses of the study pathologists were over ruled on a statistical basis without conducting a simple slide review or peer review of the data.

G) Age-related susceptibility

The Agency's review of these 12 studies provide an opportunity to determine whether pups are more sensitive than their dams to the neurotoxic effects of chemicals and whether neurotoxic effects are a more sensitive endpoint than other developmental toxicity endpoints. Developmental effect in adults (i.e., latent effects) need to be considered by the Agency. Behavioral correlates of subtle structural changes may only be seen after latencies and require more sensitive behavioral assessments than those in the DNT. As represented in the appendices of this report by a Panel member, either maternal toxicity or developmental toxicity routinely occur at comparable or lower dose levels than developmental neurotoxicity.

H) Dose selection

Some description about the rationale for the selection of dose levels for DNT should be considered.

3) Does the Panel agree with the Agency proposal to include the use of the developmental neurotoxicity study, when available, for the selection of endpoints for risk assessment of pesticides and, if so, for revision of the document: Hazard Identification: Toxicology Endpoint Selection Process to reflect this addition?

- **in acute dietary risk assessment**
- **in short-term and intermediate-term occupational and residential risk assessment**
- **in chronic dietary risk assessment**

The Panel developed consensus concerning utilization of developmental neurotoxicity studies for both new and existing pesticide registrations. For new registrations, the majority of the Panel recommended that the DNT study should be combined with the 2-generation reproduction study for compounds that are neuroactive, as discussed previously. Thus, the majority of the Panel recommended that the combination of the 2-generation reproduction and DNT study should be considered a core study for neuroactive compounds. If developmental effects are identified, additional testing could be required. For existing registrations, DNT must be included for all pesticides that are neuroactive.

The Panel agreed with the basic strategy of requiring special testing to characterize more completely the profile of developmental neurotoxic effects and to define the NOEL/LOEL for risk assessment. For this purpose, the DNT guideline was generally considered acceptable with the

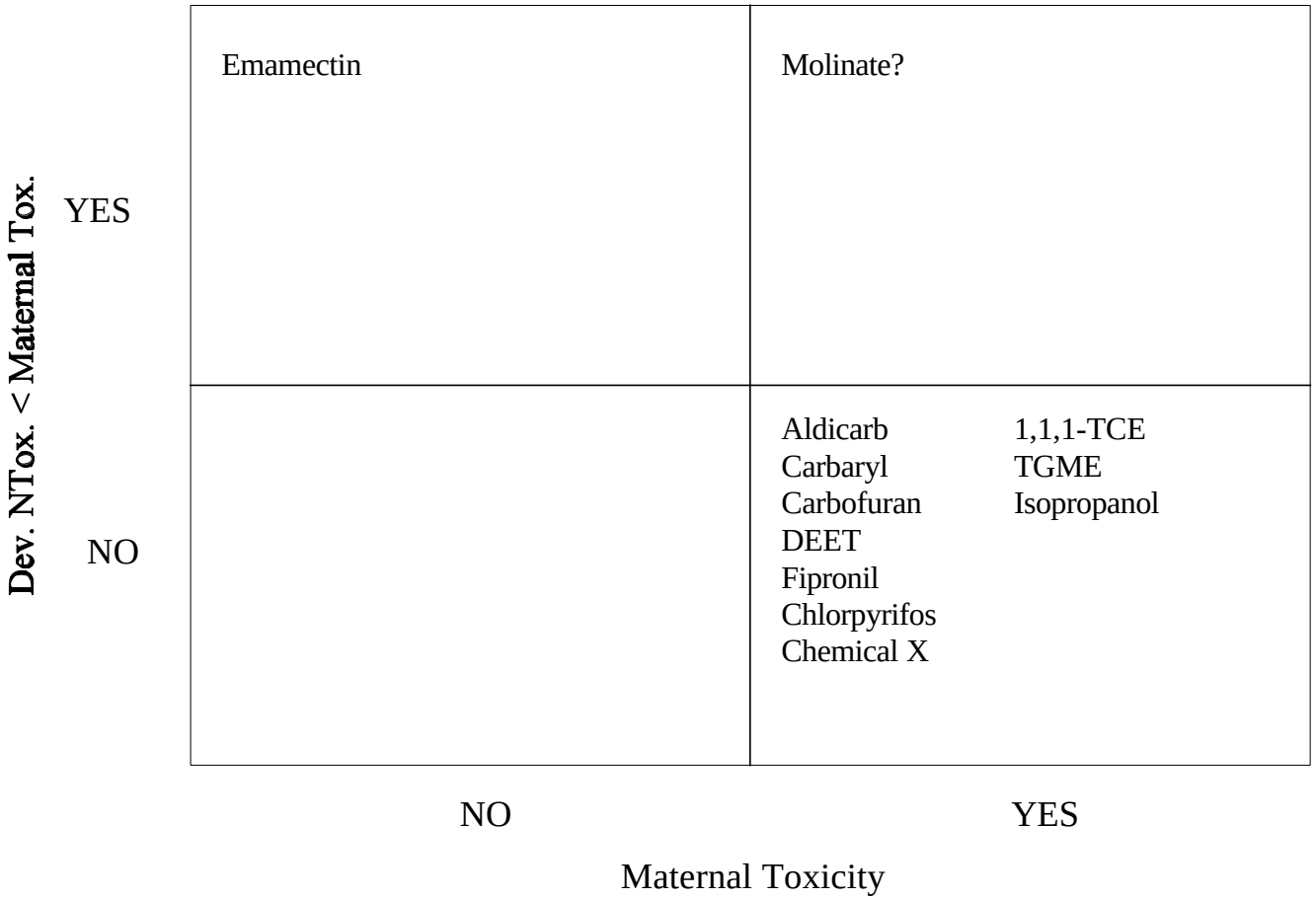
caveat that efforts by the Agency should continue to improve the DNT guideline (e.g., addressing such issues as its sensitivity, test relevance, comprehensiveness, etc).

Appendix 2. Correlation of maternal toxicity (MT), developmental toxicity (DT), and developmental neurotoxicity study (DNT) results for 12 chemicals along with feed restriction studies results.

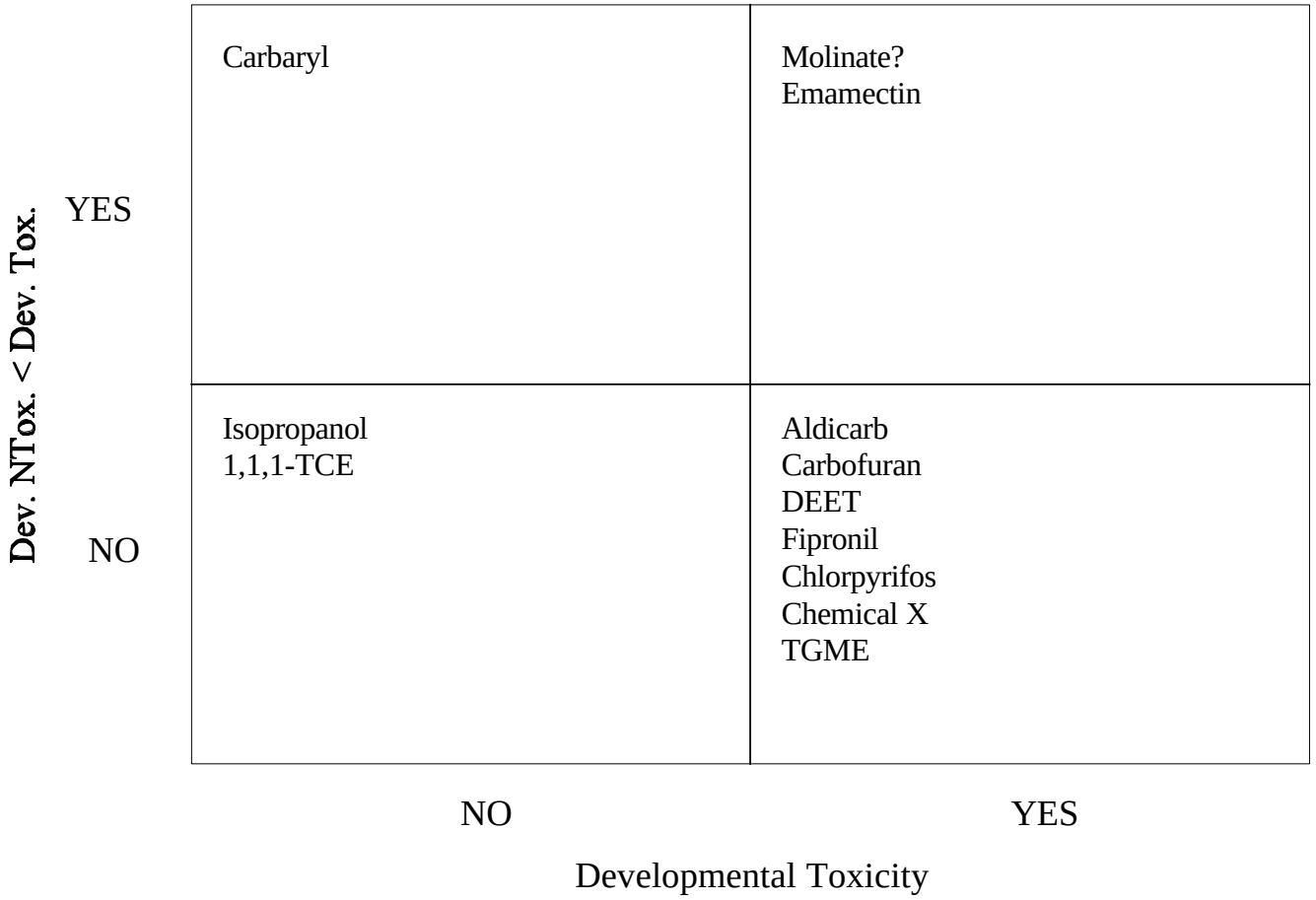
Chemical	Doses Tested (mg/kg/day)	NOEL		LOEL	Effect(s)
Aldicarb	0.05, 0.10, 0.30	MT DT DNT	0.05 0.05 0.05	0.10 0.10 0.10	↓Pl CHE ↓BW ↓MA
Carbaryl	0.01, 1.0, 10	MT DT DNT	1.0 10 1.0	10 >10 10	↓BWG, FOB, ↓CHE No effect Morphometry*
* Pathologist does not consider the morphometric changes significant. Agency review finds effects "possible" treatment related. More data requested.					
Carbofuran	1.7, 6.9, 31	MT DT DNT	1.7 1.7 1.7	6.9 6.9 6.9	↓BW, ↓FC ↓BW, ↑Mortality, Developmental delays Swimming angle dev, Y-maze time trial
Molinate	1.8, 6.9, 26.1	MT DT DNT	6.9 6.9 <1.8	26.1 26.1 1.8*	↓BW, ↓BWG, ↓FC ↓BW, ↑Mortality, Developmental delays ↓Auditory startle on day 23 pp
* Conclusion based on 2nd review. The effect was the only effect at the low dose and it occurred in only one sex at one time point at this dose level. Final decision based on weight of the evidence on Molinate and that there was a significance decrease in startle amplitude at all dose levels on day 23 postpartum.					
DEET	22.5, 90, 225	MT DT DNT	90 22.5 90	225 90 225	↓BW, ↓BWG ↓BW* ↑MA
* Table 4B shows no effects but original review reports dec. BWT at 90 and 225 mg/kg.					
Emamectin	0.1, 0.6, 3.6/2.5	MT DT DNT	3.6/2.5 0.6 0.1	>3.6/2.5 3.6/2.5 0.6	No effects ↓BW, Tremors, Limb splay, Alopecia ↓MA

Appendix 2. Correlation of maternal toxicity (MT), developmental toxicity (DT), and developmental neurotoxicity study (DNT) results for 12 chemicals along with feed restriction studies results.					
Chemical	Doses Tested (mg/kg/day)	NOEL		LOEL	Effect(s)
Fipronil	0.05, 0.9, 15*	MT DT DNT	0.9 0.05 0.9	15 0.9 15	↓BW, ↓BWG, ↓FC, Alopecia ↓BW, Developmental Delay ↓BRW, ↓Aud. Startle, ↓Swimming direction, ↓Swimming angle, ↓Y-maze time trial
* Original documents say 15, Tables 4A and 4B show 18.5.					
Chlorpyrifos	0.3, 1, 5	MT DT DNT	1** 1 1	5 5 5	↑Fasiculation, hyperactivity, hypernea ↓BW, ↓FC, ↓Survival, Development delays ↑and ↓MA, ↓BRW, ↑BR/BW%, ↑Auditory startle habituation, morphometry*
* Pathologist did not consider morphometric changes significant. Agency reviewer judged study "not acceptable" due to incomplete statistical analysis. **_CHE was observed in maternal groups given ≥0.03 chlorpyrifos.					
Chemical -X	40, 125, 400	MT DT DNT	40 125 125	125 400 400	↓FC, Salivation, Rales ↓BW ↑MA* transiently
* Table 4b shows _MA but Agency review says _MA.					
1,1,1-TCE	75, 250, 750	MT DT DNT	<750 750 750	750* >750 >750	↓BW, Altered FEP, SEP, EEG
* P.J. Spencer letter to W. Sette dated 11/19/98 of Agency Support Document.					
TGME	300, 1650, 3000	MT DT DNT	1650 300 1650	3000 1650 3000	↑Kidney wt ↓BW ↑Startle amp, ↓Startle latency*
* Agency reviewer conclusion, "The significance of these observations is not clear." as presented in Agency Support Document					

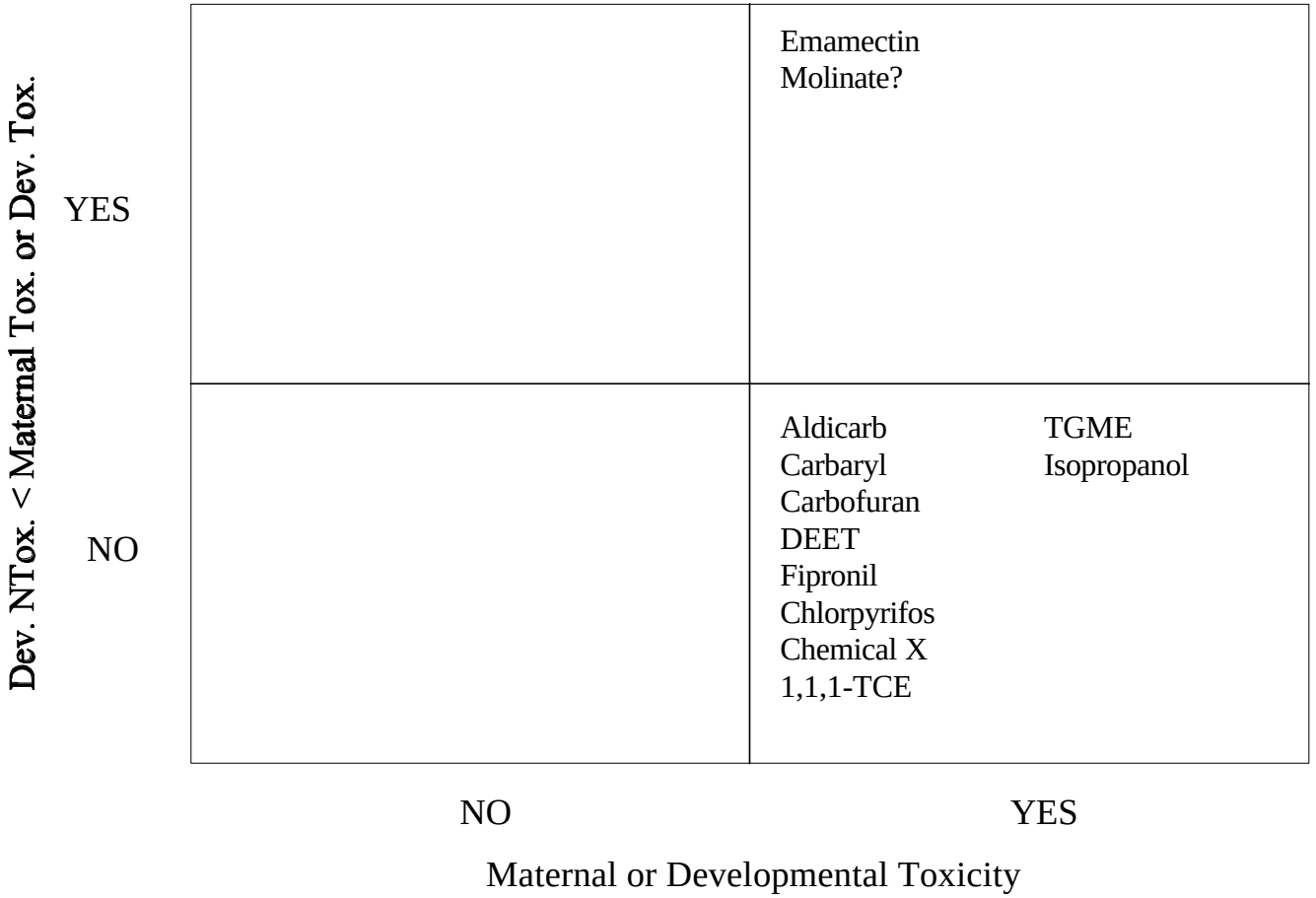
Appendix 2. Correlation of maternal toxicity (MT), developmental toxicity (DT), and developmental neurotoxicity study (DNT) results for 12 chemicals along with feed restriction studies results.					
Chemical	Doses Tested (mg/kg/day)	NOEL		LOEL	Effect(s)
Isopropanol	200, 700, 1200	MT DT DNT	700 1200 1200	1200* >1200 >1200	1/35 died
* This dose level exceeds the limit dose by 20%. One dam died.					
Diet restriction	80% of control diet	MT DT DNT			↓BW, ↓BWG ↓Vaginal opening time, ↓% Males
Diet restriction	Ad lib for 6 hr/day	MT DT DNT			↓BW, ↓BWG ↓BW, ↓Litter Wt., ↓Viability, ↓Vaginal opening time ↓BRW
* Dietary restriction studies were submitted as positive control data for Molinate. The Agency reviewer classified the study as acceptable.					



Appendix 3



Appendix 4



Appendix 5