From: Pat Kwiatkowski
To: Oshita, Cynthia@OEHHA
Subject: BCS deltamethrin letter and paper re: developmental toxicity
Date: Thursday, March 29, 2012 7:50:19 AM

Dear Cynthia,

BCS does not object to OEHHA posting the white paper by Dr. Sheets provided entitled

DELTAMETHRIN: CONSIDERATION TO LIST AS A DEVELOPMENTAL AND REPRODUCTIVE TOXICANT UNDER CALIFORNIA PROPOSITION 65

The document does not contain any information that we consider to be business confidential.

Sincerely,

Pat Kwiatkowski
Director State Regulatory Affairs and Documentation Services
Bayer CropScience LP
Telephone (919) 549 2480

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Bayer CropScience

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
Proposition 65 Implementation
P.O. Box 4010, MS-19B
Sacramento, California 95812-4010

Re: Registrant comments on OEHHA's selection of deltamethrin (52918-63-5) for the DARTIC's review for possible listing under Proposition 65

March 14, 2012

Bayer CropScience
RTP
P. O. Box 12014
RTP, NC 27709
Tel. 919 549-2000

Dear Ms. Oshita,

On January 20, 2012 a notice was issued by the Office of Environmental Health Hazard Assessment (OEHHA) indicating that OEHHA has selected deltamethrin for review by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) for possible listing under Proposition 65. Before initiating the development of hazard identification materials on deltamethrin, OEHHA has given the public an opportunity to provide information relevant to the assessment of the evidence of developmental and male and female reproductive toxicity.

Bayer CropScience (BCS), the primary registrant of the pyrethroid insecticide deltamethrin, appreciates the opportunity to have our comments reviewed before deltamethrin is considered further for possible listing under Proposition 65. BCS would like to comment on both the process that has led to deltamethrin being considered for DARTIC review as well as the overall body of reproductive toxicity data that is available and was used by DARTIC in its initial review of deltamethrin.

BCS Comment on Selection Process:

The prioritization process as presented at the July 12-13, 2011 DART IC Meeting is as follows :

Tracking Database -> Candidate Chemicals-> Chemicals Proposed for Committee Consideration-> OEHHA Selection of Chemicals for Preparation of Hazard Identification Materials

From the tracking database, candidate chemicals are identified as having some relevant DART data and some potential for exposure in California. If exposure potential is confirmed and a total of ≥ 15 reports of relevant DART

endpoints or a total of ≥ 10 reports of any single relevant DART endpoint (i.e., developmental **or** female reproductive **or** male reproductive) are identified, then the chemical is subjected to a preliminary review by DARTIC.

This selection process logic seems somewhat in line with the adage "if there is smoke, there must be fire". There are reasons for researchers performing studies on a substance that are not necessarily hazard or risk driven. In the case of deltamethrin, the number of articles that are published on the subject in part reflects the fact that, for over a decade, deltamethrin has frequently been selected for use as a model pyrethroid insecticide, having a considerable body of information in the published literature for reference.

If study/ data reliability were evaluated, there would be less than 15 reports of relevant endpoints for deltamethrin. In the case of deltamethrin, a significant number of published experiments evaluated effects of only a single dose level such that differences from control may represent biological variability rather than a treatment-related effect. Most published studies were also conducted with either an unidentified source and purity of active substance or with a formulated product, and dosing levels were not analytically verified. The undefined nature of the test material and dose formulations or use of formulations that contained 5% or less deltamethrin also represent substantive limitations for interpretation of effects. The many differences in experimental design and missing details of test procedures among studies also severely limit the opportunity to assess the biological significance or reproducibility of a given finding. Based on these facts alone, most of the articles would not meet the data reliability criteria as described by Klimisch *et al.* which are similar to the criteria recommended by U.S. EPA in the High Production Volume Program under TSCA and by EU authorities for REACH. OEHHA's selection process would be improved if a data reliability check was conducted according to criteria similar to that described by Klimisch *et al.* or recommended by U.S. EPA to assess the robustness of published experiments before recommending a DARTIC review.

Comment on the Data Reviewed by DARTIC

BCS is providing a white paper by Dr. Larry Sheets for review and consideration by OEHHA. The paper entitled

"DELTAMETHRIN: CONSIDERATION TO LIST AS A DEVELOPMENTAL AND REPRODUCTIVE TOXICANT UNDER CALIFORNIA PROPOSITION 65"

contains a detailed analysis of the data considered relevant to the reproductive toxicity of deltamethrin which we believe will prove useful to OEHHA as it considers the reproductive toxicity hazard of deltamethrin. Below is a brief summary.

The information used by pesticide regulatory authorities to evaluate deltamethrin for effects on reproduction and development includes results from Good Laboratory Practice (GLP) studies performed with laboratory animals in accordance with U.S. (EPA) and international (O.E.C.D.) test guideline requirements. This includes two- and three-generation reproduction toxicity studies in the rat, developmental toxicity studies in the rat and rabbit and a developmental neurotoxicity (DNT) study in the rat. Two of the guideline studies were not covered in the DARTIC preliminary assessment: a developmental toxicity study in the rabbit (Richard, 2001) and a developmental neurotoxicity study in the rat (Gilmore et al., 2006).

The above set of studies have been accepted to satisfy the requirements of the California and U.S. Environmental Protection Agencies, as well as by the various regulatory authorities in Canada, Europe and elsewhere, to identify potential adverse effects and establish a No-Observed-Adverse Effect Level (NOAEL). All elements of experimental design (e.g., the number of animals and dose groups, the rationale for dose selection, the route and duration of dose administration, test material purity and impurity profile, and the selection of endpoints to evaluate) for these studies are based on testing guidelines and experience that establish the suitability and reliability to identify adverse effects and to provide data suited for human risk assessments. The collective results from these guideline studies indicate that deltamethrin is not a developmental or reproductive toxicant and establish NOAELs for reproductive, maternal and developmental toxicity suitable for use in risk assessment.

As already touched upon in our comments on the selection process, in contrast, the 15 experiments reported in the open literature were not conducted according to any recognized guideline, and the many differences in experimental design and missing details of test procedures among these studies severely limit the opportunity to assess the biological significance or reproducibility of a given finding. This includes the use of different species (rats, mice and rabbits), routes of administration (dietary, gavage, subcutaneous and intraperitoneal), different exposure durations (7 to 65 days) and exposure during different life stages (e.g., adult males, pregnancy, lactation, fetal and postnatal development), with a variety of systems (e.g.,

CNS, developmental toxicity or male reproductive tissues) and different endpoints evaluated.

In BCS's opinion, the findings reported in these articles do not raise credible uncertainty regarding the potential for deltamethrin to produce developmental or reproductive toxicity. The principal findings that were reported were determined to be 1. preliminary at best and therefore would require verification, 2. non-relevant to humans or 3. not supported by results from guideline studies with technical-grade deltamethrin. The results from these guideline studies should carry considerably more weight, since they were performed under Good Laboratory Practice standards and in accordance with U.S. and International standards.

It is our scientific opinion that based upon weight-of-evidence, there is no unacceptable risk of adverse reproductive or developmental effects as a result of the currently labeled and approved agricultural and non-agricultural uses of deltamethrin. Further, Bayer CropScience does not believe the body of reliable data that exists for deltamethrin warrants further review for possible Proposition 65 listing. Bayer CropScience requests that OEHHA considers the BCS comments in this letter and the attached white paper before preparing the reproductive toxicity hazard identification materials for further DARTIC consideration. If the decision is made to proceed with a complete DARTIC review, we ask that this letter and white paper be included in the materials provided to the committee.

Sincerely,

Patricia L. Kwiatkowski PhD
Bayer CropScience LP
Director State Regulatory Affairs and Documentation Services

CC:
Christopher Reardon, Chief Deputy Director, CDPR (letter only)
Chuck Andrews, Associate Director Pesticide Program Division CDPR (letter only)
Marylou Verder-Carlos, Assistant Director Pesticide Program Division CDPR
Ann Prichard, Registration Branch Chief, Pesticide Program Division CDPR

Report Title

DELTAMETHRIN
CONSIDERATION TO LIST AS A DEVELOPMENTAL AND REPRODUCTIVE TOXICANT UNDER CALIFORNIA PROPOSITION 65

Data Requirement

None

Author

Larry Sheets, PhD, DABT

Completion Date

March 9, 2012

Sponsor

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

Report Number

US0269

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

However, these data are the property of Bayer CropScience AG and, as such, are considered to be a trade secret and confidential for all purposes other than compliance with FIFRA 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any country other than the USA.

Company:	Bayer CropScience LP
Company Agent:	Larry Sheets, Ph.D., DABT
Title:	Toxicology Fellow
Signature:	
Date:	March 9, 2012

The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The FIFRA Good Laboratory Practice Standards of 40 CFR Part 160 do not apply to the explanatory information provided in this supplemental report.

Company Agent and Report Author:
Larry Sheets

Larry P. Sheets March 9, 2012
(Date)

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I. Background and Introduction

Deltamethrin is a type II pyrethroid insecticide, with registrations in North America and around the world for pest control in homes and offices, on field crops, vegetables, fruits, potted plants and ornamentals, and to control external parasites on farm animals. Deltamethrin has been registered for such uses since 1974 and has an excellent safety record. The insecticidal activity of deltamethrin and other pyrethroids is due to disrupting the normal kinetics of sodium ion closure in the insect nervous system. The nervous system in mammals is also the most sensitive, with transient, acute neurotoxic effects evident at lower levels of exposure than doses that produce other evidence of toxicity (Soderlund et al., 2002). Since deltamethrin is rapidly degraded to inactive metabolites in vertebrate species, adverse effects are rapidly reversible after acute exposures and are not cumulative with sustained exposures.

As directed by the California Office of Environmental Health Hazard Assessment (OEHHA), the Developmental and Reproductive Toxicant Identification Committee (DART IC) performed a preliminary review of deltamethrin for possible listing under Proposition 65 due to the existence of a substantial amount of toxicological data on relevant endpoints from animal studies, in combination with the potential for human exposure (presentation and DART IC report from July 12-13, 2011 DART IC Meeting). The information reviewed by the DART IC consisted of abstracts compiled from studies reported in the open literature, along with some, but not all, of the guideline-based developmental toxicity studies performed in accordance with regulatory requirements to support product registrations. The DART IC identified 15 articles from the literature that reported evidence of adverse effects in animals – nine involving developmental endpoints and six involving male reproductive endpoints (there were none for female reproductive endpoints). Since the number of articles identified met the minimum screening criteria (15) established by OEHHA (2004) for possible listing, deltamethrin was recommended for further evaluation.

On January 20, 2012, OEHHA announced plans to prepare the reproductive toxicity hazard identification materials for deltamethrin and initiated a 60-day call-in period (ending March 20, 2012) for the public to provide comments. Bayer CropScience is providing these comments in response to the 60-day call-in period announcement. Our comments include a summary of information on developmental and reproductive toxicity from proprietary studies already reviewed and accepted by the California Department of Pesticide Regulation to support product registrations, in addition to a critical review of the open literature publications included in the DART IC preliminary review. It is our scientific opinion that based upon weight-of-evidence there is no unacceptable risk of adverse reproductive or developmental effects as a result of the currently labeled and approved agricultural and non-agricultural uses of deltamethrin. We appreciate the opportunity to have our comments reviewed before deltamethrin is considered further for possible listing under Proposition 65.

II. Database of Developmental and Reproductive Toxicity Studies

The information used to evaluate deltamethrin for effects on reproduction and development includes results from studies performed with laboratory animals in accordance with U.S. (EPA) and international (O.E.C.D.) test guideline requirements. This includes two- and three-generation reproduction toxicity studies in the rat, developmental toxicity studies in the rat and rabbit and a developmental neurotoxicity (DNT) study in the rat. Two of the guideline studies included in our assessment were not covered in the DART IC preliminary assessment: a developmental toxicity study in the rabbit (Richard, 2001) and a developmental neurotoxicity study in the rat (Gilmore et al., 2006). These studies have been accepted to satisfy the requirements of the California and U.S. Environmental Protection Agencies, as well as by the various regulatory authorities in Canada, Europe and elsewhere, to identify potential adverse effects and establish a No-Observed-Adverse Effect Level (NOAEL). All elements of experimental design (e.g., the number of animals and dose groups, the rationale for dose selection, the route and duration of dose administration, test material purity and impurity profile, and the selection of endpoints to evaluate) for these studies are based on experience that establishes the suitability and reliability to identify adverse effects and to provide data suited for human risk assessments. Furthermore, these studies were performed in compliance with Good Laboratory Practice (GLP) standards, which provide rigorous documentation suited to verify the resulting information accurately reflects the study findings.

The use of internationally-validated test guidelines to investigate effects on development and reproduction in experimental animals aims to ensure the suitability (e.g., sensitivity, reliability and reproducibility) of the data obtained in these studies. Assessing the quality of data and suitability of the testing procedures (e.g., achieve consistent results in untreated animals) includes evaluation of historical control data to demonstrate appropriately-low levels of variability within a given study and over time.

In addition, BCS is providing comments on the 15 articles the DART IC included in their preliminary review. The principal study design elements and findings from these studies are provided below, with critical comments that pertain to study design, documentation and the reported findings. Since the research cited from the literature was not based on GLP studies, there is reason and need to carefully scrutinize the study conduct and the resulting findings (e.g., the purity (and impurities) of the test chemical, the actual doses tested, the consistency or reliability of the test measures and the experience of staff performing the tests). With these uncertainties, the findings from these studies represent research that should be interpreted in the context of all available information.

A. **Guideline Studies**

1. A **three-generation reproduction study** was performed with deltamethrin. In this study, 10 male and 20 female SD rats per dose level were treated via the diet with 0, 2, 20 or 50 ppm deltamethrin technical (98% purity) (Wrenn, 1980). This study was performed before current test guidelines were established and therefore did not meet all the guideline requirements (missing parental histopathology and microscopic examination in pups was limited to F3b weanlings). Also, the highest dose level produced insufficient evidence of toxicity to satisfy the guideline requirements, with only reduced body weight at the highest dietary level (50 ppm; approximately 2.5 mg/kg/day). In order to satisfy existing guideline requirements a two-generation reproduction study was performed (Hoberman, 1992).

2. A **two-generation reproduction study** was performed in accordance with USEPA (FIFRA 83-4) guideline requirements (Hoberman, 1992). In this study, 30 male and 30 female SD rats per dose group were treated via the diet with 0, 5, 20, 80 or 320 ppm deltamethrin technical (99.7% purity) for two generations. These dietary levels resulted in doses of approximately 0, 0.4, 1.4, 5.7, or 22 mg/kg/day in P animals and 0, 0.4, 1.6, 6.2 or 26 mg/kg/day in F_1 animals, respectively. Decreased body weight gain, clinical signs consistent with a type II pyrethroid and mortality in adult and pups demonstrate the highest dietary level was a maximum-tolerated dose. The developmental NOAEL was 80 ppm, based on reduced body weight and increased pup mortality during the lactation period when exposure is especially high due to high consumption of the treated feed. There was no effect on reproduction or developmental endpoints at any dietary level. Furthermore, there was no effect on reproductive tissue weights (e.g., uterus/cervix, ovary, pituitary, testis and epididymis, seminal vesicle and coagulating gland and prostate) or microscopic lesions in male or female reproductive tissues. California DPR considered this study to be acceptable, with a reproductive toxicity NOAEL 320 ppm (corresponding to 21.2 to 24.9 (M) and 21.8 to 37.3 (F) mg/kg/day), based on no effects at the highest dietary level, and a developmental toxicity NOAEL of 80 ppm (5.8/6.7 mg/kg/day in males and females, respectively), based on decreased body weight and increased mortality at 320 ppm.

3. A **developmental toxicity study in rats** was performed in accordance with USEPA (FIFRA 83-3(a)) guideline requirements (Schardein, 1990). In this study, groups of 25 mated female Sprague-Dawley Crl:CD VAF/Plus rats were dosed by gavage with deltamethrin technical (99.2% purity) at 0, 1, 3.3, 7.0 or 11 mg/kg/day on gestation days 6 through 15, with fetuses collected by caesarean section on gestation day (GD) 20 to examine for gross, visceral and skeletal alterations. Maternal toxicity was evident at 7 and 11 mg/kg/day as death or moribund sacrifice, decreased body weight and clinical signs typical for a type II

pyrethroid. By comparison, there was no evidence of developmental toxicity in the fetus at any dose level. California DPR considered this study acceptable, with NOAELs for maternal and developmental toxicity of 3.3 and 11 mg/kg/day, respectively.

4. A **developmental toxicity study in rabbits** (Richard, 1990) was conducted with groups of 16 pregnant NZW does per group treated by gavage with deltamethrin technical (99.2%) at 0, 10, 25 or 100 mg/kg/day. The high dose of 100 mg/kg/day killed one doe (on GD 27) but produced no evidence of malformations. California DPR considered this study acceptable and determined a developmental toxicity NOEL of 25 mg/kg (based on retarded bone ossification (variation) at 100 mg/kg/day).

5. A more recent **developmental toxicity study in rabbits** was performed in accordance with USEPA (OPPTS 870.3700) and O.E.C.D. (TG 414) guideline requirements (Richard, 2001). This study was not included in the preliminary review performed by the DART IC. In this study, groups of 24 pregnant NZW rabbits per group received deltamethrin technical (99.1% purity) by gavage at 0, 3, 10 or 32 mg/kg/day from gestation days 6 to 28, with fetuses examined following caesarean section. Maternal toxicity was limited to the high dose, with decreased food consumption and body weight gain. There was no evidence of developmental toxicity (e.g., fetal mortality, developmental malformations or variations) at any dose level tested. California DPR considered this study acceptable and established a maternal toxicity NOEL of 10 mg/kg/day and a developmental toxicity NOEL of 32 mg/kg/day.

6. A **developmental neurotoxicity (DNT) study in rats** was performed in accordance with USEPA (OPPTS 870.6300) and OECD (TG 426) guideline requirements (Gilmore et al., 2006). This study was also not included in the preliminary review performed by the DART IC. This study has been reviewed and determined to be complete and acceptable by California DPR. In this study, groups of 20 Wistar rats per dose group were treated via the diet with deltamethrin technical (98.8% purity) at 0, 20, 80 or 200 ppm (1.64, 6.78 and 16.1 mg/kg/day) from GD 6 to lactation day (LD) 21. Tests used to evaluate the offspring for evidence of toxicity and developmental neurotoxicity included automated tests of motor activity, auditory startle habituation, and cognition (passive avoidance and water-maze tests for learning and memory), with gross and microscopic brain measurements and extensive microscopic analysis of peripheral and central nerve tissues for evidence of pathology. Findings at 200 ppm consisted of decreased body weight and weight gain in the dams and decreased body weight and clinical signs of toxicity during the period of exposure, with a slight delay in onset of preputial separation. The NOAEL for the maternal and developmental toxicity was 6.78 mg/kg/day.

The collective results from these guideline studies indicate that deltamethrin is not a developmental or reproductive toxicant and establish the following NOAELs for reproductive, maternal and developmental toxicity:

- Reproductive toxicity NOAEL = 24.9-27.2 mg/kg/day (the highest dose tested in the 2-generation reproduction study);
- Maternal toxicity NOAEL = 3.3 mg/kg/day (based on the developmental toxicity study in rats); and
- Developmental toxicity NOAEL of 5.8-6.78 mg/kg/day (based on decreased body weight and clinical signs in the two-generation and DNT studies); there was no evidence of teratogenic effects with deltamethrin at any dose level tested in any study.

For reference, dietary and non-occupational risk assessments are based on the most sensitive endpoint, which was a transient decrease in motor activity in adult rats, at a dose of 1.0 mg/kg. This lower NOAEL, compared to ones established for DART endpoints, is consistent with the greater sensitivity of the nervous system to transient neurotoxic effects with a pyrethroid insecticide, based on a well-established mode of action.

B. **Studies cited from the open literature**

An overview of the experimental design and principal findings from studies included in the preliminary DART IC review (May 2011 OEHHA document) is provided below. To begin, we note the following deficiencies from sound laboratory practices and toxicological principles are common to many of these studies, which generally represent basic research, with deltamethrin used as a model compound, rather than assessments suited to identify human health effects or for risk assessments:

- Single dose level: Testing only one dose level greatly limits the opportunity to judge the biological significance of the findings (e.g., evaluate biological variation / consistency among dose groups and a trend associated with dose level (dose-response));
- Uncertain reliability (consistency) of the measures: Authors generally report all differences from control as treatment-related effects, without determining whether those results exceed the range of biological variability among control groups (historical control) or whether a finding is dose related or can be repeated (robustness);
- Undefined treatment / Unverified doses: It is unknown what treatment the animals received, as there is no information on purity and impurities in the test materials or analysis of the dose formulations to verify doses (e.g., concentration, homogeneity and stability). Some studies tested commercial formulations with only 2.8% or 5% deltamethrin (95-97% undefined formulating agents).

1. **Neurochemical / Neurobehavioral**

A persistent increase in the expression of cytochrome P450 (CYP) in the offspring liver and brain was reported in a study by **Johri and coworkers (2006a,b)** following treatment of pregnant rats from gestation day 5 to 21 with deltamethrin (purity not specified and doses not verified) at dose levels of 0.25, 0.5 or 1.0 mg/kg/day. These changes are consistent with the induction of enzymes involved in the metabolic degradation of deltamethrin in rats. The reported persistence of this finding is unexpected, and requires verification, since CYP activity is typically reversible after treatment is discontinued. Furthermore, this effect would not likely be relevant to man, since man metabolizes deltamethrin primarily via carboxylesterases (hCE1 and hCE2), rather than CYP enzymes. Therefore, these findings are not relevant or of concern to human health and do not provide evidence of developmental or reproductive toxicity.

In an abstract for a scientific meeting, **Richardson and co-workers (2004)** reported evidence of increased markers of dopaminergic neurons (dopamine transporter, vesicular monoamine transporter 2 and tyrosine hydroxylase) in the rat striatum. In this study, 3 mg/kg deltamethrin (purity not specified and doses not verified) administered orally to female C57BL/6J mice at 3-day intervals (from 2 weeks prior to breeding until day 21 of lactation) was associated with changes in dopaminergic neurochemistry in the offspring 7 days after treatment was discontinued. Findings in this study were not associated with a change in dopamine or its metabolites DOPAC and HVA. Limitations of this study include uncertainty whether these differences from the concurrent control group (one treated group compared to one control group) represent an effect or are within the range of biological variability for untreated mice. It is also unclear whether these findings would occur in rats or other species and whether such findings represent adaptive changes in response to a relatively high dose or are reversible. Moreover, these results were presented at a meeting in 2004 and have never been published. Therefore, these results represent very preliminary information that requires verification.

Lazarini and co-workers (2001) report effects on swimming behavior, motor activity and striatal dopamine levels in rats exposed during gestation to 0.08 mg/kg deltamethrin (purity not specified, doses not verified). As in the previous study, the testing of only one dose level limits assessment of whether the reported differences between one treated group and a control group represent biological variability or treatment-related effects. Information from the guideline developmental neurotoxicity (DNT) study reviewed above determined that treatment with deltamethrin (98.8% purity) at doses higher dose levels (1.64, 6.78 or 16.1 mg/kg/day) and longer duration (GD6 to PND 21) had no effect in a swimming test of cognitive function (M-maze test on PND 60 and 67) or automated measures of motor activity (PND 13, 17, 21 or 60) (Gilmore et al.,

2006). Therefore, these findings differ from those reported in the guideline DNT study, which we consider to provide a much more definitive assessment of motor function and cognition.

Aziz and co-workers (2001) report evidence of impaired cognitive performance from prenatal exposure of Wistar rats to 1 mg/kg of **2.8% technical-grade deltamethrin in an emulsifiable concentrate** (2.8 EC; doses were not verified) on gestation days 14-20 (the "brain growth spurt" in rats), with the offspring tested 6 and 12 weeks after parturition. Findings attributed to treatment consisted of a modest (16-28%) increase in acetylcholinesterase activity and a moderate (39-48%) decrease in muscarinic receptor (^{3}H-QNB) binding in the hippocampus, with a slight difference in "re-learning" performance in the Y-maze. The effects reported to occur with this 2.8 EC formulation suggest a persistent effect on cognitive function, but require verification without the formulating agents (which constitute >95% of the formulation), under conditions better suited to judge the relationship with treatment at defined dose levels. Moreover, as indicated above, deltamethrin technical was determined to have no effect on two tests of cognition in the guideline DNT study (water maze and passive avoidance), with a much longer period of exposure (GD 6-PND 21) at higher dose levels (Gilmore et al., 2006).

In conclusion, the potential effects of deltamethrin on the developing nervous system reported in these articles are either not suited to assess toxicity in man or provide only preliminary information that requires verification. In the meantime, these findings are not supported by results from the guideline developmental neurotoxicity study, which was performed in compliance with GLP and in accordance with internationally-accepted standards for use to identify potential hazards and provide data suited for risk assessments.

2. Developmental Toxicity

In a series of studies performed at the U.S. Environmental Protection Agency, deltamethrin was determined to produce no evidence of developmental toxicity in rats or mice, even at dose levels that produced marked maternal toxicity. In studies performed to investigate developmental toxicity (teratogenicity), **Kavlock and co-workers (1979)** administered deltamethrin technical (technical-grade from Roussel Uclaf, France, doses not analyzed) by gavage to CD-1 mice at doses of 0, 3.0, 6.0, or 12.0 mg/kg/day on GD 7-16 or to Sprague-Dawley rats at doses of 0, 1.25, 2.5 or 5.0 mg/kg/day on GD 7-20. Findings at high doses included maternal toxicity (salivation or convulsions after daily dosing and decreased weight gain) without fetal toxicity or evidence of skeletal or visceral abnormalities in fetuses at any dose level in either species. The noteworthy strengths of this work include the use of technical-grade material provided by a

reliable source, the use of three dose levels in each species with a robust sample size (15 per dose level), and providing sufficient details for the reader to understand the procedures and interpret the test results. Moreover, the results from these studies are consistent with results from the guideline developmental and reproductive toxicity studies performed with technical-grade deltamethrin, as described above.

Abdel-Khalik and co-workers (1993) tested a 5% formulation of deltamethrin (95% undefined formulating agents) for developmental toxicity in rats. In this study, doses of 1, 2.5 or 5 mg/kg **Decis 5 Flow formulation** (doses not verified) were administered orally to female rats from gestation day 6 to 15, with fetal examinations on GD 19. Since early embryonic deaths and excessive growth retardation occurred at all dose levels, all dose levels were too toxic for use to evaluate effects on development. The principal findings (soft-tissue anomalies) in the survivors are associated with overt fetal toxicity, rather than indicative of a developmental or teratogenic effect. Moreover, this formulation (with 5% active ingredient) is much more toxic than the technical-grade deltamethrin tested in guideline studies, including the rat developmental toxicity study; therefore, this study is not suitable to evaluate the toxicity of deltamethrin. As described above, the guideline rat developmental toxicity study demonstrated no evidence of fetal or developmental toxicity, even at maternally-toxic doses of 7 and 11 mg/kg/day, that were associated with mortality or moribund sacrifice, decreased body weight and clinical signs typical for a type II pyrethroid (Schardein, 1990). Since the toxicity in the present study was excessive at all three dose levels and certainly involved toxicity from the formulating agents, this study is not suited to evaluate the potential for deltamethrin to produce developmental toxicity.

Husain and Seth (1991) report results from a study with deltamethrin (purity not specified, doses not analyzed) for developmental toxicity, with doses administered by gavage at 7 mg/kg/day to pregnant rats on gestation days 5 to 21 and to post-weaning rats on postnatal days 22 to 37. Pups treated during gestation had reduced birth weight and growth rate, with delayed onset of certain reflexes and developmental landmarks, and decreased monoamine oxidase, ATPase and AChE activities and decreased polyamine levels in brain areas. By comparison, rats treated on PND 22-37 were reported to have the opposite effect on monoamine oxidase and a "differential effect" on polyamine levels in brain areas. The reported decrease in birth weight and weight gain in this study, along with information from other studies with gavage administration, indicate animals received a very toxic dose and evidence of delayed development would most likely be secondary to decreased body weight during development. By comparison, dietary levels that achieved a higher dose in the guideline developmental neurotoxicity study produced no evidence of developmental delay, based on measures of motor activity ontogeny, auditory startle habituation and sexual maturation (Gilmore et al., 2006). The available information suggests

the differential findings with prenatal and postnatal treatment that are reported in this article represent biological variability (no treatment effect in either group), rather than a differential effect associated with exposure during different stages of development. Limitations to the study reported in this article include the testing of only one dose, the selection of a dose that produced decreased birth weight and post-natal growth (which confound interpretation) and the undetermined reliability of the measures. Given these limitations and uncertainties, along with the high dose at which these findings were reported, this article does not provide information that is useful to understand the potential for deltamethrin to produce developmental toxicity.

Badaeva and Nedorenko (1991) investigated the effect of deltamethrin on placenta and cardiac tissue of the progeny (article written in Russian). In this study, pregnant rats received oral doses of deltamethrin (identified as "decis") at 1/20 or 1/100 the LD50 dose level "during the critical periods of neonatal development". The principal findings were evidence of ultrastructural and histochemical changes in the placenta and cardiac tissue of the progeny. Too little information is provided for a critical review, including no description of the test material or dose levels that were used and there were no other measures to evaluate toxicity, for reference. It is also unclear whether these findings would impair placental or cardiac function or would be evident with more common measures, including cardiac weight and histopathology (which have not been seen in guideline studies with deltamethrin), or would be reversible. In the absence of sufficient details or confirmatory evidence from other studies, this should be considered only preliminary information that requires verification.

3. Male reproductive toxicity

Andrade and co-workers (2002) report decreased absolute (not relative) testis and epididymis weights and decreased diameter of seminiferous tubules in male offspring of female rats treated with deltamethrin (98.8% purity, doses not verified) by gavage at 0, 1.0, 2.0 and 4.0 mg/kg/day from gestation day 1 to lactation day 21. Maternal and reproductive outcome and landmarks of male sexual development were assessed. Fertility, sexual behavior, and multiple reproductive endpoints (e.g., organ weights, sperm evaluations, testosterone concentration, and testicular histology) were examined on the adult male offspring. Maternal toxicity was not evident at any dose level. Findings attributed to deltamethrin were limited to decreased absolute (not relative) testis and epididymis weights and diameter of seminiferous tubules at 4.0 mg/kg deltamethrin, which were acknowledged to be subtle changes. While occurrence at the highest dose supports these representing treatment-related effects, these endpoints were determined to not be affected in a guideline 2-generation reproduction study (Hoberman, 1992), with a longer period of exposure at dietary

levels that achieved doses of 24.9 mg/kg/day. Therefore, we consider these findings to either be unreliable or of no concern, given the subtle nature of these findings and occurrence at a high dose of 4.0 mg/kg/day (no effect at 2.0 mg/kg), and the fact that the guideline 2-generation reproduction study identified no similar effects at higher doses with longer durations of exposure.

Abdallah and co-workers (2010) report effects on sperm parameters in male mice (10/group) treated by gavage with 5 mg/kg deltamethrin (purity not specified, doses not analyzed) for 21 days, with measures of body weight, testis and epididymis weights and sperm parameters evaluated at term. Findings in this study were decreased sperm count, motility and viability and increased morphologically-abnormal spermatozoa, with no effect on testis or epididymis weight. It is noteworthy that an organophosphorus insecticide (dimethoate) was determined to produce the same profile of effects. These findings are of no concern, given the use of mice (rat is the preferred species for a reproductive toxicity study), of undefined age and weight range, insufficient details about the test material, dose verification and test procedures, and testing only one dose level. Moreover, the animals were not treated for a full cycle of spermatogenesis (43 days in mice) and these findings occurred at a relatively high dose. Therefore, the results from this study represent basic research that is not suited for purposes of human safety and risk assessment.

Issam and co-workers (2009) investigated potential oxidative stress with deltamethrin as a representative type II pyrethroid. In this study, deltamethrin (purity not specified, doses not analyzed) was given by **subcutaneous administration** to male Wistar rats (6/dose group) for 30 days at 0.003 mg/kg/day, for 30 days at 0.003 mg/kg/day, followed by 15 days at 0.03 mg/kg/day (treated for 45 days), or for 30 days at 0.003 mg/kg/day, followed by 15 days at 0.03 mg/kg/day and 15 days at 0.3 mg/kg/day (treated for 60 days), with measures of testis histopathology, sex hormones and oxidative stress at termination. Findings included decreased sperm production with apoptotic figures in the testis and cell fragments in the seminiferous tubule lumen, with decreased FSH, LH and testosterone. The article concluded "an arrest of spermatogenesis, a significant disharmony in sex hormones and MDA levels" related to dose and length of treatment, with involvement of lipid peroxidation. Notable limitations and uncertainties include the test material content and relevance of route of administration to human circumstances of exposure and risk assessments. Furthermore, the internal dose via subcutaneous administration is unknown but likely much higher than via oral administration. Therefore, these results would require confirmatory evidence to understand the relevance to assessing male reproductive toxicity.

El-Gohary and co-workers (1999) performed a study to examine testicular apoptosis with deltamethrin and the potential protective effect of a nitric oxide synthase inhibitor. The authors conclude the results of this study provide evidence of testicular apoptosis in rats treated with 1 mg/kg deltamethrin (purity not specified, doses not analyzed) **via intraperitoneal administration (in corn oil)** for 21 days. Microscopic examination of testis showed apoptosis confined to basal germ cells, primary and secondary spermatocytes. Notable limitations include treatment via a route of uncertain relevance to human exposures (by-pass metabolism in the gut and first-pass metabolism by the liver), testing of a single dose and lack of characterization of the test material and doses. One could only speculate that 1 mg/kg i.p. would produce much higher tissue levels than with oral administration (the kinetics of absorption or clearance in the corn oil matrix were not determined). Moreover, the histopathology reported in the testis was not evident in the testis from P or F1 rats in the guideline two-generation reproduction study, with exposure through a full cycle of spermatogenesis at higher dose levels given via the diet (Schardein, 1990). Therefore, the results from this study represent basic research that is not suited for purposes of human safety and risk assessment.

Decreased testis, seminal vesicle and prostate weights, with decreased testosterone, sperm counts and fertility were reported by **Abd el-Aziz and co-workers (1994),** who treated male rats by gavage with 1 or 2 mg/kg/day **Decis 5 Flowable formulation** (purity estimated 5% of the formulation, doses not analyzed) for 65 days. This formulation was reported to contain only 5% deltamethrin, with the balance (95%) undefined. The toxicity expressed in this study was much greater than was evident in guideline studies with technical-grade deltamethrin, which is consistent with substantial toxicity associated with one or more of the formulating agents. Also, the animals were reported to be maintained on an unusual diet of milk and barley and the sample sizes (5/group for organ weights and 8/group for mating) were insufficient to make reliable determinations of effects on tissue weight and fertility. Questions are also raised by an organophosphorus insecticide (diazinon; identified only as a 50% oily solution) producing the same profile of effects. For reference, the guideline two-generation reproduction study determined technical-grade deltamethrin had no effect in P or F1 males on testis or prostate weight or histopathology for these three tissues or fertility at higher doses and a longer duration of exposure (Hoberman, 1992). Therefore, the findings in this study are either unreliable or reflect toxicity from the formulating agents and are therefore not suited to evaluate male reproductive toxicity with deltamethrin.

Salem and co-workers (1988) report slight effects on reproductive endpoints in rabbits (3 per group) treated with deltamethrin (purity not specified, doses not analyzed) by oral administration in gelatin capsules for six weeks at doses of 1/10 and 1/100 the LD50 (doses otherwise undefined). Specifically, the authors report "indications of decreased libido" for "the majority of the treated animals", a slight decrease in ejaculate volume, a decrease in sperm concentration and increased abnormal and dead sperm. Similar findings were reported with the organophosphorus insecticide dimethoate. These findings represent preliminary research results that would require confirmation, with a grossly-insufficient sample size, the use of the rabbit (rat is the preferred species for a reproduction study), no details of test material composition and no determination of dose levels or verification of the doses, with insufficient results provided in the report.

Overview: The studies reported in all of these articles have severe limitations that would require verification to support reliable evidence of an effect on male reproduction. Four studies cite an effect on sperm production; however, the collective results are not consistent and do not challenge the determinations from the guideline multi-generation reproduction studies. Specifically, evidence of decreased testis and epididymis tissue weights and histopathology of testis, epididymis and seminal vesicle were not evident in P and F1 males in the guideline study. The guideline two-generation reproduction study **(Hoberman, 1992)** provides a much more comprehensive and definitive assessment of reproductive toxicity with deltamethrin. Strengths of this guideline study include the use of a model that is accepted for use to evaluate reproductive toxicity, exposure that extends through the entire cycle of spermatogenesis, the use of a well-characterized test material and verified doses, including a maximum-tolerated dose and a NOAEL, and the use of internationally-accepted standards (GLP and test procedures) for study conduct. The guideline two-generation reproduction study has established that treatment with deltamethrin beginning 10-weeks prior to mating the P-generation had no effect on reproduction in P- or F1-generation rats and treatment that continued until terminal sacrifice did not affect male reproductive tissues (e.g., testis, epididymis, prostate, or seminal vesicles with coagulating glands), with doses as high as 22 mg/kg/day. Males in the guideline study are exposed for 10 weeks before mating (for exposure throughout a complete cycle of spermatogenesis) and the microscopic examination of testis and epididymis includes a detailed examination of sperm morphology through all stages of maturation. Finally, the guideline study represents exposure of the F1 progeny beginning prior to conception through sexual maturation to produce an F2 generation – any finding in P-generation animals would also be evident or more severe in the F1 progeny used to produce the F2 generation. The articles from the literature provide no information that should affect the determination from the guideline studies that deltamethrin is not toxic to the male reproductive system.

III. Summary Assessment: Developmental and Reproductive Toxicity

The collection of 15 articles from the preliminary review by the DART IC is considered representative of research in the open literature to investigate the potential effect of deltamethrin on reproduction and development. The number of articles that are published on the subject reflect the fact that deltamethrin is often selected for use as a model for research on pyrethroid insecticides, with a considerable body of information in the published literature for reference. The many differences in experimental design and missing details of test procedures among these studies limit the opportunity to compare results to assess the biological significance or reproducibility of a given finding. This includes the use of different species (rats, mice and rabbits), routes of administration (dietary, gavage, subcutaneous and intraperitoneal), different exposure durations (7 to 65 days) and exposure during different life stages (e.g., adult males, pregnancy, lactation, fetal and postnatal development), with a variety of systems (e.g., CNS, developmental toxicity or male reproductive tissues) and different endpoints. Another limitation of the studies from the open literature is the common use of only one dose level, such that differences from control may represent biological variability rather than a treatment-related effect. Without a supporting database to provide a measure of biological variability in control animals, such findings should be considered preliminary evidence that requires confirmation. Finally, the undefined nature of the test material and dose formulations or use of formulations that contained 5% or less deltamethrin also represent substantive limitations for interpretation and comparison.

In conclusion, the findings reported in these articles were determined to not raise credible uncertainty regarding the potential for deltamethrin to produce developmental or reproductive toxicity. The principal findings reported in these articles were determined to be preliminary and require verification, non-relevant to humans or to not be supported by results from guideline studies with deltamethrin. The results from the guideline studies for developmental and reproductive toxicity should carry considerably more weight than the research articles, since they were performed under GLP conditions and in accordance with U.S. and International standards accepted for use to investigate developmental and reproductive toxicity and for risk assessments. This collection of guideline studies has been accepted by the state of California, the USEPA and other regulatory authorities around the world to establish that deltamethrin is not a developmental or reproductive toxicant. The results from these guideline studies are suited to identify potentially-adverse effects at relatively high doses and to provide data suited for use in risk assessments to establish safe levels of exposure. Based on all the available evidence, it is appropriate to conclude that deltamethrin should not be listed under Proposition 65 as a developmental or reproductive toxicant.

IV. References

Guideline Studies

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