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Re: **Endocrine Disruptor Screening Program Tier 1 Screening Order Issuing Announcement (74 FR 54422); EPA–HQ–OPP–2009–0634**

The accompanying comments are being submitted on behalf of the more than two million members and supporters of People for the Ethical Treatment of Animals and the Physicians Committee for Responsible Medicine who are concerned about promoting reliable and relevant toxicity testing strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals. Our comments are submitted in response to issuance of Tier 1 Screening Orders for the Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP) for the first seven chemicals, issued on October 29, 2009, under the request for existing data and "other scientifically relevant information" (OSRI) in which "persons other than recipients" have 90 days to respond.

Introduction

EPA has initiated the EDSP Tier 1 screening for the first group of 67 chemicals by issuing test orders from October 29, 2009, through February 26, 2010. The 67 Phase I chemicals consist of 58 pesticide active and nine High Production Volume (HPV) chemicals used as pesticide inert ingredients (also known as "pesticide inerts"). These chemicals were chosen for testing based on exposure potential considering four exposure pathways for each type of chemical. The four exposure pathways identified for pesticide active ingredients are: food, drinking water, residential use, and occupational exposure. The four exposure pathways identified for HPV/pesticide inert chemicals are: human biological monitoring, ecological biomonitoring, drinking water, and indoor air.[1]

These chemicals are to be tested in five *in vitro* and six *in vivo* assays (**Table 1**). The stated purpose of the Tier 1 battery is to "identify substances that have the potential to interact with the EAT [estrogen/androgen/thyroid] hormonal systems…".[2] The EPA has stated that it intends to use a weight-of-evidence approach to evaluate the results of the Tier 1 studies,[3] and based on this assessment, EPA will determine which, if any, of the Tier 2 tests are necessary. The putative Tier 2 battery consists of developmental and reproductive toxicity tests in several vertebrate species and is designed to identify and establish dose-response relationships for any adverse endocrine-related effects.

These comments address the test orders for carbaryl,[4,5] a widely used insecticide that has been extensively tested as part of registration. This testing involves dozens of toxicity tests in

vertebrate animals, including reproductive and chronic/lifecycle studies in rodents, fish and birds, as well as metabolism and pharmacokinetics studies.[6] These tests kill thousands of animals and include many of the same endpoints addressed in the presumptive EDSP Tier 2 tests (**Table 2**).

In its letter to EPA approving the Information Collection Request, OMB instructed EPA to "promote and encourage test order recipients to submit Other Scientifically Relevant Information (OSRI) *in lieu* of performing all or some of the Tier I assays, and EPA should accept OSRI as sufficient to satisfy the test orders to the greatest extent possible." In the interest of increasing the efficiency of the EDSP, the comments before provide existing data and OSRI in support of these OMB instructions to EPA, focusing on animal testing and vertebrate testing in particular. There is one section for each of the seven chemicals; references follow each section.

In all cases, the equivalent of Tier 2 (reproductive toxicity in one or more generations) information is available for rodents and in some cases also for fish and birds. There are two primary reasons for carrying out Tier 1 testing: 1) to discern mechanistic information about a chemical (i.e. does it function by interacting with the E, A or T hormone system) and 2) to evaluate what, if any, Tier 2 testing is warranted. Thus, if Tier 2 data already exist for a chemical, there is very little rationale for performing Tier 1 testing.

EPA has not articulated how endocrine disrupting chemicals would be regulated based on mechanism of action. Even though there is no precedence for such regulation to date, future regulation may benefit from mechanism of action information; in fact such information is critical for reduced dependency on whole animal testing and for improving the accuracy of hazard and risk determination as outlined in the 2007 NRC report: Toxicity Testing for the 21st Century: a Vision and a Strategy.[7] Rather than using whole animal tests such as the uterotrophic or Hershberger simply because they are available, mechanistic information can be obtained through non-animal means, in binding, transcriptional activation, or other cell-based systems, some of which are in use by the EPA's ToxCast™ program. A more efficient structure for the EDSP would be to start with a series of mechanistic *in vitro* assays to determine which, if any, of the endocrine pathways a chemical interacts with, and target any further testing accordingly.

The EPA's ToxCast™ program profiled 56 of the 73 EDSP Phase I chemicals in 14 assays directly related to endocrine activity (including estrogen, androgen, thyroid, and aromatase), and in an expanded set of 78 high throughput assays, including nuclear receptor and CYP450-related assays.[8] The advantage of the structure of the ToxCast™ program's database is that connections can rapidly be made between *in vitro* assay results and existing mammalian and ecotoxicity data, which greatly facilitates identification and interpretation of mechanism of action information.

Preliminary results from Phase I of the entire ToxCast™ program are promising.[9] Linkages between high-throughput *in vitro* results and *in vivo* endpoints can be made, and potency rankings for groups or classes of chemicals are also being explored. Intriguingly, high "activity" across a large number of molecular pathways correlates inversely with lowest observed effect level (LOEL) in mammalian studies.

Rather than a default application of the full battery of Tier 1 assays to data-rich chemicals such as pesticides, a more efficient and potentially more useful approach would be to evaluate the existing relevant data, reproductive and developmental information in particular, in combination with information from a series of *in vitro* mechanistic assays such as those included in the Tier 1 and in ToxCast™, to determine what, if any, further testing is warranted.

References

[1] 74 FR 17579. April 15, 2009; EPA Final List of Initial Pesticide Active Ingredients and Pesticide Inert
Ingredients to be Screened Under the Federal Food, Drug, and Cosmetic Act.

[2] 74 FR54415, October21, 2009. Endocrine Disruptor Screening Program (EDSP); Announcing the
Availability of the Tier 1 Screening Battery and Related Test Guidelines; Notice.

[3] Response to Comments on the Public Review Draft of the Information Collection Request (ECR) entitled "Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP)", contained in Docket ID no. EPA-HQ-OPPT-2007-1081, page 16.

[4] 74 FR 54422, October 21, 2009; Endocrine Disruptor Screening Program; Tier 1 Screening Order Issuing Announcement, Order Issuance Schedule.

[5] EPA Endocrine Disruptor Screening Program, status of EDSP Orders/DCIs (http://www.epa.gov/endo/pubs/edsp_orders_status_012810.pdf; accessed 3 February 2010)

[6] 72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional Chemicals.

[7] NRC (Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies Press, Washington, DC. Available at: http://www.nap.edu/catalog.php?record_id=11970. Accessed 25 January 2009.

[8] Kavlock et al. (2009) Biological Profiling of Endocrine Related Effects of Chemicals in ToxCast™. Poster presentation available at http://www.epa.gov/NCCT/toxcast/files/summit/40P%20Kavlock%20TDAS.pdf. Accessed February 4, 2010.

[9] Judson et al. (2009) "The Toxicity Data Landscape for Environmental Chemicals" Environmental Health Perspectives, Volume 117, Number 5, May 2009 (http://ehp.niehs.nih.gov/members/2008/0800168/0800168.pdf, accessed 5 February 2010).

Table 1: EDSP Tier 1 Assays

	Species	Mechanism addressed	Endpoints	suggested equivalent information
in vitro				
ER TA: OPPTS 890.1300 OECD TG 455	endogenous human ERα	Estrogen agonists	ERa-dependent transcriptional activation	effect ovary/uterus size, histology, male/female fertility
ER binding OPPTS 890.1250	Rat uterine cytosol	Estrogen agonists, antagonists	ER binding	effect ovary/uterus size, histology, male/female fertility
AR binding: OPPTS 890.1150	rat prostate cytosol	Androgen agonists, antagonists	AR binding	effect on testes size, histology, male/female fertility
Steroidogenesis - H295R OPPTS 890.1550	human	Steroid synthesis (estrogen and testosterone)	testosterone, estrogen hormone levels	effect on estrogen/testosterone levels, sex organs, male/female fertility
Aromatase OPPTS 890.1200	human	Aromatase inhibition, the enzyme responsible for the conversion of androgens to estrogens	$^3H_20$ released during the conversion of androstenedione to estrone	effect on estrogen/testosterone levels, sex organs, male/female fertility
In vivo:				
Uterotrophic OPPTS 890.1600 OECD TG 440	rat, mouse immature: PND 18 - 21 ovarectimized: 6 - 8 weeks	Estrogen agonists, antagonists (in GD, not well developed)	body weight, uterine weight, optional: histolopathology of vagina	evidence of estrogenic activity, uterine or vaginal weight changes, uterine or vaginal histology, effects on fertility reproduction
Hershberger OPPTS 890.1400 OECD TG 441	rat, mouse	Androgen agonists, antagonists, and 5α-reductase inhibitors	ventral prostate (VP), seminal vesicle (SV), levator ani-bulbocavernosus (LABC) muscle, paired Cowper's glands (COW) and the glans penis (GP)	evidence of androgenic activity, male sex organ weights or histology, effects on fertility reproduction

Pubertal female OPPTS 890.1450	rat	Anti-thyroid, estrogenic or anti-estrogenic (including alterations in receptor binding or steroidogenesis), luteinizing hormone, follicle stimulating hormone, prolactin or growth hormone levels or via alterations in hypothalamic function	Growth (daily body weight), Age and body weight at vaginal opening, Organ weights: Uterus, Ovaries, Thyroid, Liver, Kidneys, Pituitary, Adrenals. Histology: Uterus, Ovary, Thyroid, Kidney. Hormones: Serum thyroxine (T4), Serum thyroid stimulating hormone (TSH). Estrous cyclicity: Age at first estrus, length of cycle, percent of animals cycling. Standard blood panel, including creatinine and blood urea nitrogen.	evidence of estrogenic or thyroid activity, uterine or vaginal weight changes, uterine or vaginal histology, effects on fertility reproduction
Pubertal male OPPTS 890.1500	rat	Anti-thyroid, androgenic, or anti-androgenic [androgen receptor (AR) or steroid-enzyme-mediated], alterations in gonadotropins, prolactin, or hypothalamic function	Growth (daily body weight), Age and body weight at preputial separation, Organ weights: Seminal vesicle plus coagulating gs, Ventral prostate, Dorsolateral prostate, Levator ani/bulbocavernosus muscle complex, Epididymides, Testes, Thyroid, Liver, Kidneys, Pituitary, Adrenals. Histology: Epididymis, Testis, Thyroid, Kidney. Hormones: Serum testosterone, Serum thyroxine (T4), Serum thyroid stimulating hormone (TSH). Standard blood panel, including creatinine and blood urea nitrogen.	evidence of androgenic or thyroid activity, male sex organ weights or histology, effects on fertility reproduction
Amphibian metamorphosis OPPTS 890.1100	*Xenopus laevis*	hypothalamic-pituitary-thyroid (HPT) axis, Androgen agonists, antagonists, testosterone synthesis	Day 5: developmental assessment: hind limb and body length, body weight, developmental stage. Day 21 (termination): Developmental stage, SVL, hind limb length and wet body weight, thyroid gland histology.	evidence of androgenic or thyroid activity, male sex organ weights or histology, effects on fertility reproduction
Fish short-term reproductive screen OPPTS 890.1350 OECD 229	fathead minnow	hypothalamus-pituitary-gonadal (HPG) axis	survival, reproductive behavior, secondary sexual characteristics (number and size of nuptial tubercles), gonadal histopathology, gonado-somatic index, plasma concentrations of vitellogenin, 17β-estradiol and testosterone, fecundity (# eggs/female), fertility (%embryos/eggs)	evidence of estrogenic/androgenic activity, effects on fertility of reproduction

Table 2: Pesticide Data requirements related to EDC

Toxicological data requirements			**Use**	
OPPT guideline		**Relevant endpoints**	**food**	**non-food**
870.4100	Chronic oral: rodent	12 months exposure: gross necropsy plus histopathology of liver, kidneys, adrenals, testes, epididymides, ovaries, uterus, thyroid (with parathyroid), spleen, brain	R	CR
870.6200	90-day neurotoxicity	FOB: autonomic function (lacrimation, salvation, etc), convulsions, tremors, abnormal motor movements, reactivity to general stimuli (no reaction to hyperreactivity), general level of activity (unresponsive to hyperactive), posture and gait abnormalities, forelimb and hindlimb grip strength, foot splay, sensorimotor responses, body weight, neuropathology.	R	R
870.4200	Carcinogenicity	24 month exposure: clinical observations, blood smears, gross necropsy, possible histopathology of salivary glands, esophagus, stomach, intestine, liver, pancreas, gallbladder, brain, pituitary, peripheral nerve , spinal cord , eyes, adrenals, parathyroid, thyroid, trachea, lungs, pharynx, larynx, nose. aorta, heart, bone marrow, lymph nodes, spleen, kidneys, urinary bladder, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries, female mammary gland, all gross lesions and masses, skin.	R	CR
870.3700	Prenatal developmental toxicity, rat and rabbit	Exposure througout gestation: fetal deaths, resoption, sex and weight of each fetus, skeletal and soft-tissue abnormalities of fetuses	R	R
870.3800	Reproduction and fertility	Standard 2-gen: integrity and performance of the male and female reproductive systems, including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, and on the growth and development of the offspring. **P animals:** Cycling in females, sperm count, morphology, motility in males. Organ weights: uterus , ovaries, testes, epididymides, seminal vesicles, prostate, brain, pituitary, liver, kidneys, adrenal glands, spleen. Hisotpathology of vagina, uterus with oviducts, cervix, and ovaries, testis, epididymis, seminal vesicles, prostate, coagulating gland, pituitary and adrenal glands. **F1:** weight and gross abnormalities throughout developement, age of vaginal opening and preputial separation, anogenital distance, same organ weights as P, same histopath as P. **F2 weanlings:** histopathological examination of treatment-related abnormalities.	R	R
870.6300*	Developmental neurotoxicity	Perinatal exposure. Pup weight during growth, gross developmental abnormalities, motor activity, learning and memory, neuropathology (brain)	R	CR
870.7800*	Immunotoxicity	Functional tests: either antibody plaque-forming cell (PFC) assay or ELISA-based antibody reaction, NK cell activity. Cell counts of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations.	R	R

Terrestrial and aquatic non-target organism data requirements			Use				
			terrestrial	aquatic	forestry	residential	Greenhouse/ indoor
850.2300	Avian reproduction	Eggs laid, percent fertilized, eggs not cracked, shell thickness, hatching, chick survival	R	R	R	R	NR
850.1400 (OECD TG 210)	Fish early life stage (freshwater)	Exposure of eggs until hatching: cumulative mortality, numbers of healthy fish at end of test, time to start of hatching and end of hatching, numbers of larvae hatching each day, length and weight of surviving animals, numbers of deformed larvae, numbers of fish exhibiting abnormal behavior.	R	R	R	R	NR
850.1500	Fish life cycle	Locomotion, behavioral, physiological, and pathological effects, spawning, egg numbers, fertility, and fecundity.	CR	CR	CR	CR	NR

*new in 2007

Carbaryl, CAS number 63-25-2
Test order numbers: EDSP-056801-57 through 60
Test order date: November 12, 2009

I. Introduction

Carbaryl, most commonly known as Sevin, is an N-methyl carbamate (NMC) insecticide, which was first registered in 1959 for use on cotton (EPA, 2008a). It is the most frequently used NMC insecticide (Mathew et al., 1995), applied to fruits, vegetables, forage, cotton and many other crops because of its low toxicity and relatively short lifetime in the environment (Peterson et al., 1994).

The NMC pesticides share a common mechanism of toxicity by affecting the nervous system via acetylcholinesterase (AChE) inhibition. AChE is an enzyme that breaks down acetylcholine and terminates its stimulating action in the synapse between nerve cells and target cells. When AChE is inhibited, acetylcholine builds up, prolonging the stimulation of the target cell. This excessive stimulation potentially results in a broad range of adverse effects on many bodily functions. (EPA, 2008b)

In March 2005, the U.S. Environmental Protection Agency (EPA) issued generic and product-specific data call-ins (DCIs) for carbaryl. The generic DCI required toxicology studies, worker exposure monitoring, and environmental fate data. The product DCI required acute toxicity and product chemistry data for all pesticide products containing carbaryl. In response to the 2005 DCIs, many carbaryl registrants chose to voluntarily cancel their carbaryl products. Approximately 80% of all of the carbaryl end-use products registered at the time of the 2003 Interim Reregistration Eligibility Decision (IRED) have since been canceled (EPA, 2008a).

II. Existing Toxicological Data Related to Endocrine Disruption

Reproductive and developmental toxicity

In its Environmental Health Criteria monograph, the International Programme on Chemical Safety (IPCS) states that carbaryl has been shown to affect mammalian reproduction and perinatal development adversely in a number of species. However, with the exception of a small number of studies, all adverse reproductive and developmental effects were noted only at doses that caused overt maternal toxicity (ICPS, 1994). More recent studies described below in rats, including the first thyroid study, were summarized in a 2001 addendum to this report (Van Apeldoorn et al., 2001).

In a ***two-generation reproductive toxicity study***, groups of 30 Sprague-Dawley CD rats of each sex were fed diets containing carbaryl at a concentration of 0, 75, 300 or 1500 ppm (equal to 0, 4.7, 24 and 93 mg/kg bw per day for males and 0, 4.8, 21 and 96 mg/kg bw per day for females) for both generations. Signs of systemic toxicity were seen in F0 and F1 parents at 300 and 1500 ppm. Treatment had no effect on mating, fertility, pregnancy or gestational indices, numbers of implants,

live or dead pups per litter, or on the per cent post-implantation loss. In addition, no treatment-related gross or histological lesions were seen in the reproductive organs of either sex in any generation. In F1 pups, effects on the lactational index and mortality rate were observed at the highest dietary concentration, but these effects were not statistically significant. Vaginal opening and preputial separation were delayed at the highest dietary concentration, but these effects were judged to be related to lowered body weights. F2 pups showed dose-related decreases in 4-day survival and lactational index. While these trends were statistically significant, no statistical significance was reached at 300 or 1500 ppm. No treatment-related malformations were observed. ***The NOAEL for parental toxicity based on effects on body and liver weights in parental animals was 75 ppm, equal to 4.7 mg/kg per day. The NOAEL for toxicity in the offspring, based on increased F2 pup mortality, was also 75 ppm (Tyl et al., 2001).***

In a ***developmental toxicity study, 25 pregnant Sprague-Dawley Crl:CD (SD) BR rats*** received carbaryl by gavage at a dose of 0, 1, 4 or 30 mg/kg bw per day on days 6–20 of gestation. No deaths were observed. Body-weight gain and food consumption were significantly reduced among dams at 30 mg/kg bw per day. There were no treatment-related effects on pre- or post-implantation loss, number of fetuses per litter or fetal sex ratio. Fetal body weights were significantly reduced at the highest dose. The fetal and litter incidences of incomplete or absent ossification of the seventh cervical centrum, incomplete ossification of the fifth sternebra and non-ossification of the first metacarp were increased at 30 mg/kg bw per day, a maternally toxic dose. There were no treatment-related changes in the incidences of malformations. ***The NOAEL for maternal toxicity was 4 mg/kg bw per day, on the basis of salivation and reduced body-weight gain and food consumption. The NOAEL for embryo- and fetotoxicity was 4 mg/kg bw per day, on the basis of decreased body weight and delayed ossification (Repetto-Larsay, 1998).***

In another ***developmental toxicity study, groups of 22 pregnant New Zealand white rabbits*** received carbaryl (purity, 99%) in a 0.5% an aqueous solution of methylcellulose by gavage at a dose of 0, 5, 50 or 150 mg/kg bw per day on days 6–29 of gestation. The body-weight gain of does at the highest dose was reduced. There were no treatment-related effects on pre- or post-implantation loss, the number of fetuses per litter or the fetal sex ratio. Fetal body weights were significantly reduced at 150 mg/kg bw per day. There was no treatment-related change in the incidence of malformations. ***The NOAEL for maternal toxicity was 5 mg/kg bw per day, on the basis of decreased erythrocyte cholinesterase activity at 50 mg/kg bw per day. The NOAEL for embryo- and fetotoxicity was 50 mg/kg bw per day, on the basis of reduced fetal body weight per litter (Tyl et al., 1999).***

Tests similar to those proposed for EDSP Tier 2 have already been conducted for carbaryl and NOAELs for embryo- and fetotoxicity and have been established in multiple mammalian species. Adverse reproductive and developmental effects were generally observed only at doses that also produced maternal toxicity. Since Tier 1 tests are designed to identify chemicals for further testing in Tier 2, new Tier 1 tests are unnecessary.

Thyroid effects

In a 90-day subchronic toxicity study, groups of five male Sprague-Dawley rats, were fed diets containing carbaryl at a concentration of 0, 250, 1500 or 7500 ppm (equivalent to 0, 12, 75 and 380 mg/kg bw per day) for 90 days. Decreased growth was seen for animals at 1500 and 7500 ppm. ***Carbaryl induced follicular-cell hypertrophy of the thyroid at concentrations greater than 250 ppm (Totis, 1997).***

Additional reproductive and developmental toxicity (fish, amphibians and birds)

Carbaryl affected developing zebrafish *(Danio rerio*) embryos by retarding stage progression based on the appearance of stages in development and had a significant effect on embryo size from the time the eggs were laid until they hatched. Embryos were smaller than the controls even at the lowest concentration tested (1/1000 carbaryl/aged tap water). Embryos in the highest concentration developed more slowly and hatched later than the controls and eggs in other dilutions. The average mortality rate was low, indicating that carbaryl does not directly kill embryos at the concentrations tested (Todd et al., 2002).

In a modified ***Frog Embryo Teratogenesis Assay-Xenopus (FETAX)***, embryotoxic effects of carbaryl were evaluated by exposing *X. laevis* embryos to 1, 2, 4, 8, 16 and 24mg/Lcarbaryl from stage 8 to stage 47. From an estimated LC50 of 20.28mg/L and TC50 of 8.43 mg/L a TI of 2.41 was derived, indicating that ***carbaryl is teratogenic for X. laevis embryos***. The most characteristic malformation, classified as abnormal tail flexure, involved a significant percentage of larvae from 1 mg/L carbaryl and higher, reaching 100% at 24 mg/L carbaryl. Histopathological screening revealed tail musculature and notochord as the main targets for carbaryl. This axial–skeletal damage was hypothesized to be related both to the inhibition of AChE, with consequent muscular tetanic spasms, and to disorders in the organization of the connective tissue matrix surrounding the notochord (Bacchetta et al., 2008).

In bobwhite quail (*Colinus virginianus*) fed diets containing carbaryl at 0, 300, 1000, or 3000 mg/kg for 22 weeks, no adverse effects were observed in any factors related to hatchability, post-hatching viability, or gross pathology of newly hatched birds (Fletcher and Leonard, 1986a). In mallard ducks (*Anas platyrhyncos*), these researchers reported that the 3000 mg/kg diet level was toxic with some lethality, decreased numbers of eggs, and thinner egg shells. No effects were seen at the 300 or 1000 mg/kg diet levels (Fletcher and Leonard, 1986b).

Data already exist in amphibians and fish for EDSP Tier 1 endpoints and for avian endpoints to be addressed in proposed Tier 2 tests. These data indicate that while carbaryl adversely affects development in amphibians and fish, its effects in amphibians are secondary to AChE inhibition.

Additional endocrine effects (fish)

Carbaryl induced thyroid dysfunction and changes in circulating thyroid hormones in walking catfish (*Clarias batrachus*). Carbaryl exposure at12 mg/liter for 96 hr decreased thyroxine (T4) but increased triiodothyronine (T3) and the T3/T4 ratio in serum. In the pharyngeal thyroid, this exposure increased T4 as well as peroxidase activity and decreased T3 and the T3/T4 ratio while in the posterior kidney, it decreased T4 and T3, peroxidase activity and the T3/T4 ratio. Carbaryl exposure at 5 mg/liter for 16 days decreased T4 and T3 and the T3/T4 ratio in serum. In the anterior kidney, this exposure increased T4 and peroxidase activity but decreased T3 and the T3/T4 ratio, while in the posterior kidney, it decreased T3 and the T3/T4 ratio and stimulated extrathyroidal conversion of T4 to T3 (Sinha et al., 1991).

Statistically significant reductions in gonadotropic hormone in the pituitary gland and plasma in green snakeheads (*Channa punctatus*) were observed following exposure to carbaryl at a concentration of 1.66 mg/litre. Gonadotropic hormone levels continued to decrease with continued exposure, with a 30% decrease in the pituitary gland and a 50% decrease in serum after 30 days of exposure (Ghosh et al., 1990).

Effects in humans

In a study designed to investigate the association between environmental exposure to carbaryl and altered semen quality in adult men, subjects (n = 272) were recruited through a Massachusetts infertility clinic and individual exposures were measured as spot urinary concentrations of 1-naphthol (1N), a metabolite of carbaryl. Compared with men in the lowest 1N tertile, men in both the medium and high 1N tertiles were more likely to have below-reference sperm concentration and sperm motility. The sperm motion parameter most strongly associated with 1N was straight-line velocity (Meeker et al., 2004). A subsequent study by these researchers confirmed that 1N from carbaryl, rather than naphthalene, exposure is likely responsible for the observed association between 1N and sperm motility (Meeker et al., 2007).

***Effects* in vitro**

In a transcriptional activation assay for human estrogen and human progesterone receptor (hER and hPR) activity in human breast and endometrial cancer cells, carbaryl alone weakly activated estrogen- and progesterone-responsive reporter genes and decreased estradiol- and progesterone-induced reporter gene activity. In whole-cell competition binding assays, the carbaryl demonstrated a limited capacity to displace radiolabeled estrogen or progesterone from hER or hPR (Klotz and Arnold, 1997). The authors concluded that carbomyl displays a generalized endocrine activity that is not mediated via receptor binding.

In a transcriptional activation assay for the thyroid hormone receptor (TR) signaling pathway, carbaryl and its metabolites 1-naphthol and 2-naphthol showed antagonist activity with IC50s of 8.40×10−5 M, 7.62×10−5M and 7.73×10−5 M, respectively. No significant differences among the

antagonist activities of these three chemicals were observed. Neither carbaryl nor its metabolites activated TR (Sun et al., 2008)

III. Summary and Recommendations

Carbaryl has been widely used for more than 60 years and its mechanism of toxicity via acetylcholinesterase (AChE) inhibition has been thoroughly characterized. Moreover, tests similar to those required under Tier 1 and proposed for Tier 2 of the EDSP have been recently conducted in a number of vertebrate species. These include developmental toxicity tests in amphibians and fish, a two-generation reproductive toxicity test in rats, and developmental toxicity tests in rats and rabbits. In addition, thyroid endpoints have been addressed in a subchronic toxicity test in rats and effects on thyroid hormone levels have been measured in fish. Relevant data in humans and *in vitro* is also available. Since the existing data adequately address EDSP endpoints, no further testing of carbaryl is necessary.

IV. References

Bacchetta R, Mantecca P, Andrioletti M, Vismara C, Vailati G. Axial-skeletal defects caused by Carbaryl in Xenopus laevis embryos. 2008. *Sci Total Environ.* 392(1):110-8.

Fletcher DW, Leonard MI. 1986a. Toxicity and reproduction study with carbaryl technical in Bobwhite quail. Neillsville, Wisconsin, Bio-Life Associate Ltd (Report No. 85-DR-15). Rhône-Poulenc Agro. Lyon, France.

Fletcher DW, Leonard MI. 1986b. 28-day dietary toxicity and reproduction pilot study with carbaryl technical in Mallard ducks. Neillsville, Wisconsin, Bio-Life Associate Ltd (Report No. 85 DR P14). Rhône-Poulenc Agro. Lyon, France.

Ghosh P, Bhattacharya S, Bhattacharya S.1990. Impairment of the regulation of gonadal function in Channa punctatus by metacid 50 and carbaryl under laboratory and field conditions. *Biomed Environ Sci*. 3(1): 106-112.

International Programme on Chemical Safety. 1994. Environmental Health Criteria 153: Carbaryl. World Health Organization. Geneva, Switzerland. Available at: www.inchem.org/documents/ehc/ehc/ehc153.htm.

Klotz DM, Arnold SF, McLachlan JA. 1997. Inhibition of 17 beta-estradiol and progesterone activity in human breast and endometrial cancer cells by carbamate insecticides. *Life Sci.* 60(17):1467-75.

Mathew L, Reddy MLP, Rao TP, Iyer CSP, Damodaran AC. 1995. Simple spectrophotometric method for the determination of carbaryl in soil and insecticide formulations. *Analyst.* 120:1799–801.

Meeker JD, Ryan L, Barr DB, Herrick RF, Bennett DH, Bravo R, Hauser R. 2004. The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality. *Environ Health Perspect.* 112(17):1665-70.

Meeker JD, Barr DB, Serdar B, Rappaport SM, Hauser R. 2007. Utility of urinary 1-naphthol and 2-naphthol levels to assess environmental carbaryl and naphthalene exposure in an epidemiology study. *J Expo Sci Environ Epidemiol*. 17(4):314-20.

Peterson HG, Boutin C, Martin PA, Freemark KE, Ruecker NJ, Moody MJ. 1994 Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. *Aquat Toxicol*. 28:275–92.

Repetto-Larsay M. 1998. Carbaryl developmental toxicology study in the rat by gavage. Unpublished report No. SA 98070. Rhône-Poulenc Agro, Centre de Recherche, Sofia Antipolis Cedex, France.

Sinha, N, Lal, B, Singh, TP. 1991. Carbaryl-induced thyroid dysfunction in the freshwater catfish Clarias batrachus. *Ecotoxicol. Environ. Saf.* 21: 240–247.

Sun H, Shen OX, Xu XL, Song L, Wang XR. 2008. Carbaryl, 1-naphthol and 2-naphthol inhibit the beta-1 thyroid hormone receptor-mediated transcription in vitro. *Toxicology.* 249(2-3):238-42.

Todd NE, Van Leeuwen M. 2002. Effects of Sevin (carbaryl insecticide) on early life stages of zebrafish (Danio rerio). *Ecotoxicol Environ Saf.* 53:267-72.

Totis M. 1997 Investigation of the metabolism of [^{14}C]-carbaryl in the 15 month old rat following dietary administration. Unpublished report No. SA 95288. Rhône-Poulenc Ag Co. North Carolina, USA.

Tyl RW, Marr MC, Myers CB. 1999. Developmental toxicity evaluation (with cholinesterase assessment) of carbaryl administered by gavage to New Zealand white rabbits. Unpublished report No. 65C-7297-200/100. Research Triangle Institute. Research Triangle Park, North Carolina, USA.

Tyl RW, Myers CB, Marr MC. 2001. Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD® (Sprague-Dawley) rats. Unpublished report No. 65C-07407-400. Research Triangle Institute. Research Triangle Park, North Carolina, USA.

United States Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances. 2008a. Amended Reregistration Eligibility Decision (RED) for Carbaryl. Available at: www.epa.gov/oppsrrd1/REDs/carbaryl-red-amended.pdf.

United States Environmental Protection Agency (EPA). 2008b. Carbaryl; Order Denying NRDC's Petition to Revoke Tolerances. Federal Register. 73(210):64229-64246.

Van Apeldoorn ME, Wolterink G, Turkstra E, van Raaij MTM, van Hoeven-Arentzen PH, van Engelen JGM. 2001. Toxicological evaluations, Carbaryl (addendum). *Pesticide residues in food 2001*. Centre For Substances and Risk Assessment, National Institute of Public Health and the Environment. Bilthoven, The Netherlands. Available at: http://www.inchem.org/documents/jmpr/jmpmono/2001pr02.htm

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