FULFILLING DATA REQUIREMENTS FOR THE US EPA ENDOCRINE DISRUPTOR SCREENING PROGRAM USING EXISTING AND NONTRADITIONAL DATA

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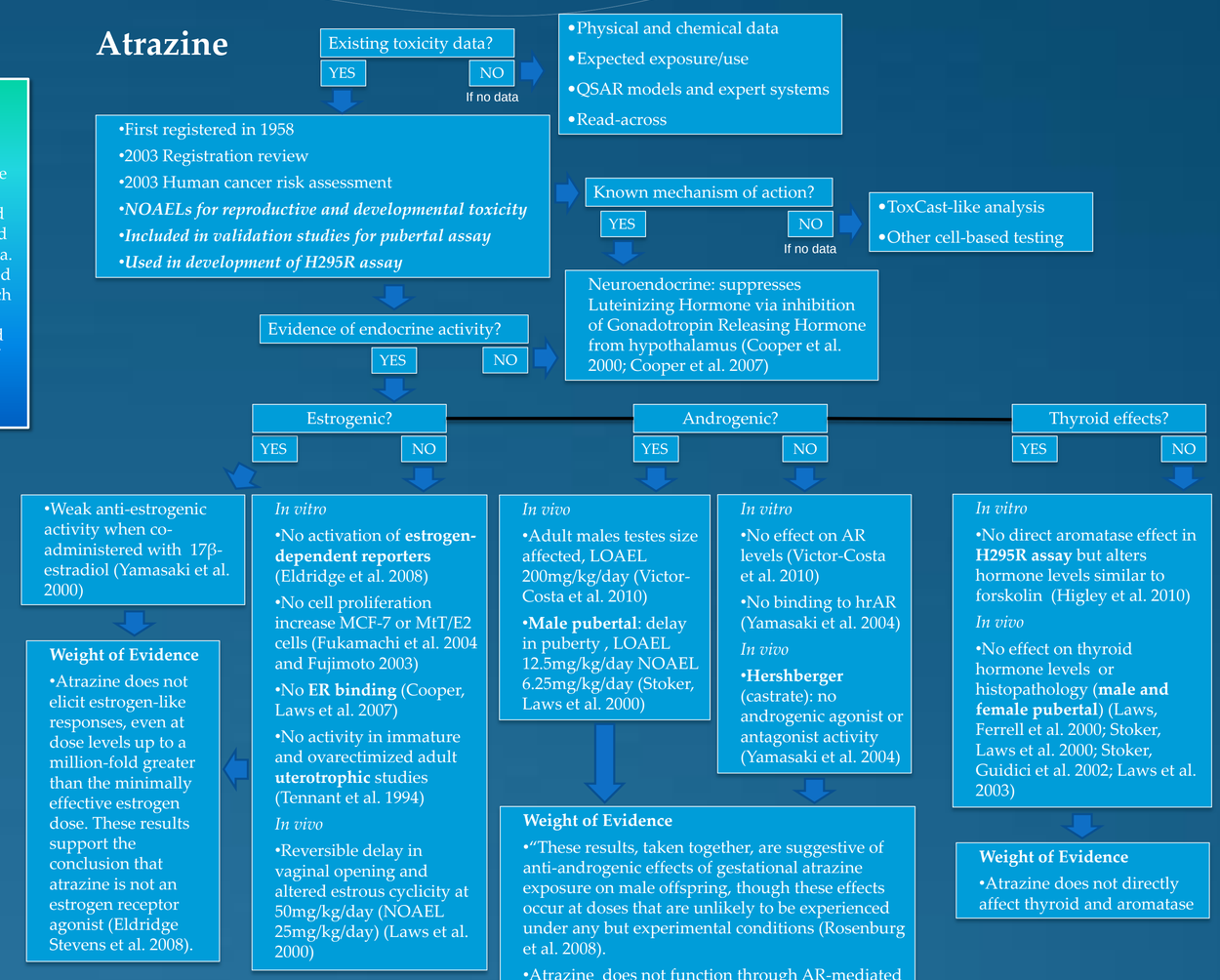
ABSTRACT

New methods of generating and evaluating toxicity data for chemicals are needed to cope with the increasing demands of new testing programs. One such approach involves the use of existing data combined with non-testing and testing strategies to create a custom, integrated testing strategy (ITS) based on the specific properties of a chemical. ITS can be applied to new or existing programs to increase efficiency and save animals and resources. We recently proposed an ITS approach to the U.S. EPA Endocrine Disruptor Screening Program (EDSP), which the EPA launched in October 2009 by issuing orders for the first 67 chemicals to be tested in the EDSP Tier 1 battery of assays. As a condition of approving EPA's plan to collect data for the program, the Obama Administration (Office of Management and Budget) instructed the EPA to promote and encourage the use of Other Scientifically Relevant Information (OSRI) *in lieu* of performing some or all of the Tier 1 assays. The Phase I chemicals consist of 58 pesticide active ingredients and nine High Production Volume (HPV) pesticide inert chemicals, all of which have been subjected to dozens of toxicity tests, often including reproductive and chronic/lifecycle studies in rodents, fish and birds. Many of these chemicals also have information available from mechanistic *in vitro* assays that supplement *in vivo* information already available. Building on our previous work using ITS principles, we show that OSRI-based arguments are sufficient to satisfy Tier 1 data requirements for a number of Phase 1 chemicals. This approach can be used to address the endocrine disrupting potential of chemicals in any regulatory context. In fact, development of a Guidance Document for the use of Test Guidelines for assessing endocrine disruptors within the context of existing information is a current high priority project at the Organization for Economic Cooperation and Development.

USE OF EXISTING INFORMATION TO DESIGN AN INTEGRATED STRATEGY FOR HAZARD ASSESSMENT

The EPA has been instructed by OMB to use Other Scientifically Relevant Information (OSRI) for the first 67 chemicals to be tested in Phase I; these chemicals are data-rich and many have existing Tier 1 and/or Tier 2 data. Here we show that existing data can be used within the context of an integrated approach provides information that is sufficient for assessing the hazard of these chemicals and additional testing using official "validated" Tier 1 tests would not necessarily provide additional information.

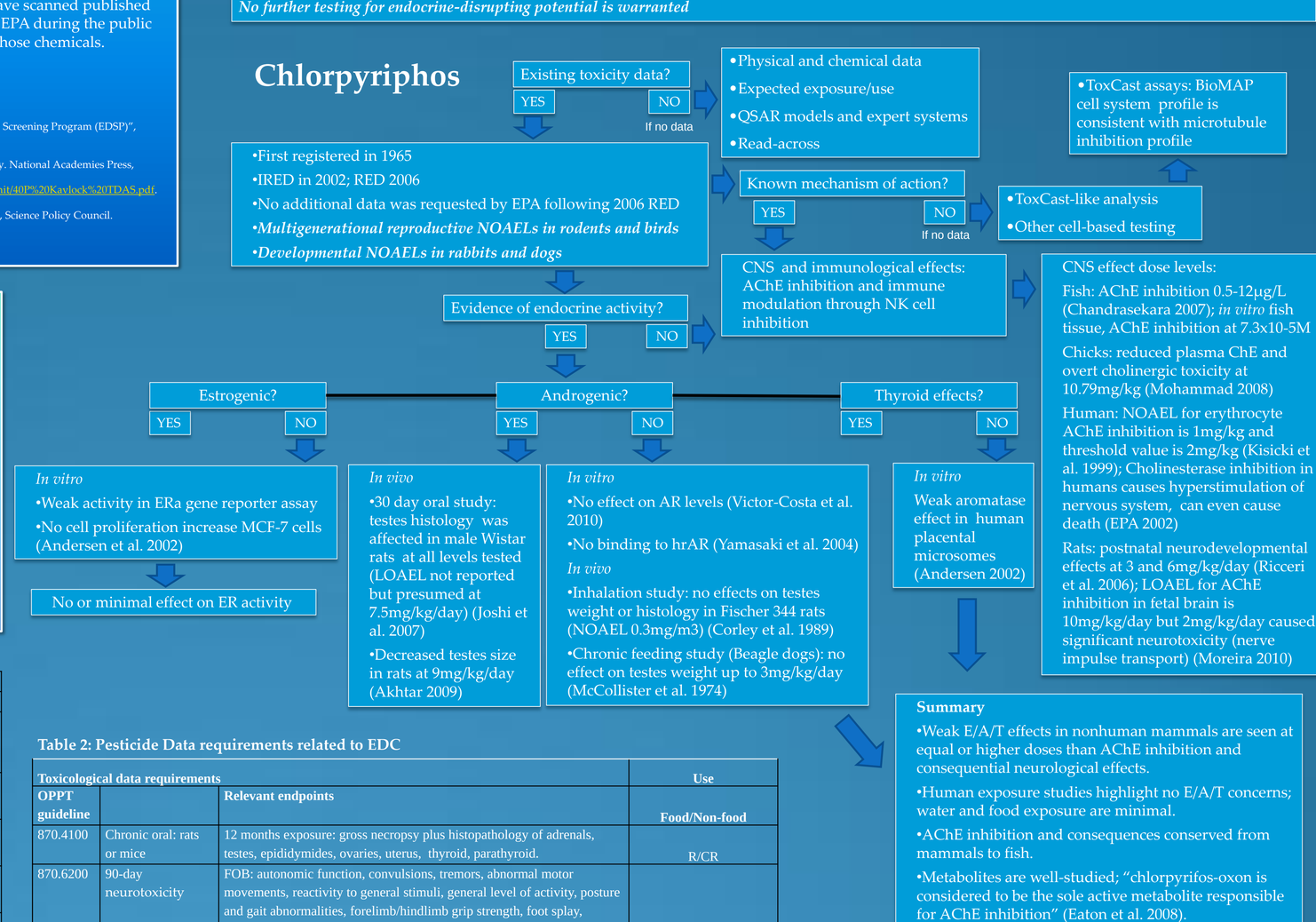
Atrazine



Conclusion:

•Atrazine indirectly affects the endocrine system through the central nervous system and has consequential effects on development and reproduction in rodents , the LOAELs and NOAELs for which have been established.
•Atrazine does not bind or activate the estrogen receptor *in vitro* or *in vivo*.
•Atrazine does not affect AR binding or activation.
•Atrazine does not affect thyroid hormone-dependent processes in rodent or in amphibians.
•Atrazine does not appreciably affect development or sexual differentiation in amphibians or fish.
•For most endpoints included in the Tier 1 tests, LOAEL and/or NOAELs have been established.
•Atrazine has been thoroughly tested in a wide range of vertebrate species using a variety of methods, including protocols similar if not identical to those required under Tier I EDSP and several under Tier II as well.
No further testing for endocrine-disrupting potential is warranted

Chlorpyriphos



Summary

•Weak E/A/T effects in nonhuman mammals are seen at equal or higher doses than AChE inhibition and consequential neurological effects.
•Human exposure studies highlight no E/A/T concerns; water and food exposure are minimal.
•AChE inhibition and consequences conserved from mammals to fish.
•Metabolites are well-studied; "chlorpyrifos-oxon is considered to be the sole active metabolite responsible for AChE inhibition" (Eaton et al. 2008).
Data gaps (such as estrogen and androgen receptor modulating capacity) may be easily filled using in vitro assays in Tier I and/or in ToxCast.

INTRODUCTION

EPA's Endocrine Disruptor Testing Program (EDSP) is organized into two tiers of tests; 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…". The EPA has stated that it intends to use a weight-of-evidence approach to evaluate the results of the Tier 1 studies, 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.

The EDSP Tier 1 consists of five *in vitro* and six *in vivo* assays (**Table 1**). Conducting all of the proposed eleven EDSP tests would use a minimum of 520 animals and cost between $324,000 and $938,000 per chemical.

The EPA has initiated the EDSP by issuing testing orders for the first 67 chemicals consisting of 58 pesticide active and nine High Production Volume (HPV) chemicals used as pesticide inert ingredients. Since these chemicals are among the most thoroughly tested of all chemicals, much data already exists, some of which may be relevant to establishing the endocrine disrupting potential of these chemicals. For registration, pesticides are subjected to extensive testing, including reproductive and chronic/lifecycle studies in rodents, fish and birds, as well as metabolism and pharmacokinetics studies. These tests kill thousands of animals and include many of the same endpoints addressed in the presumptive EDSP Tier 2 tests (**Table 2**). In all cases, the equivalent of some Tier 2 (reproductive toxicity in one or more generations) information is available for rodents and in some cases also for fish and birds. Since 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, if Tier 2 data already exist for a chemical, there is very little rationale for performing Tier 1 testing.

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."

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. This approach would also be consistent with both the 2007 NAS Report, Toxicity Testing in the 21st Century, as wells EPA 's 2009 Strategic Plan for Evaluating the Toxicity of Chemicals .

In the interest of increasing the efficiency and reducing the numbers of animals used to satisfy Phase I of the EDSP, we have scanned published literature and publically available databases to provide OSRI for 12 Phase I chemicals. We submitted this information to EPA during the public commenting period and here present this data, along with an ITS for assessing endocrine disrupting potential of two of those chemicals.

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.
74 FR54415, October21, 2009. Endocrine Disruptor Screening Program (EDSP); Announcing the Availability of the Tier 1 Screening Battery and Related Test Guidelines; Notice.
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.
72 FR 60934, October 26, 2007: EPA 40 CFR Parts 9 and 158: Pesticides; Data Requirements for Conventional Chemicals.
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.
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%20TDA5.pdf. Accessed February 4, 2010.
US Environmental Protection Agency. 2009. The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals. Office of the Science Advisor, Science Policy Council. Washington, DC. Available at : http://www.epa.gov/osa/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf. Accessed 25 August 2010.

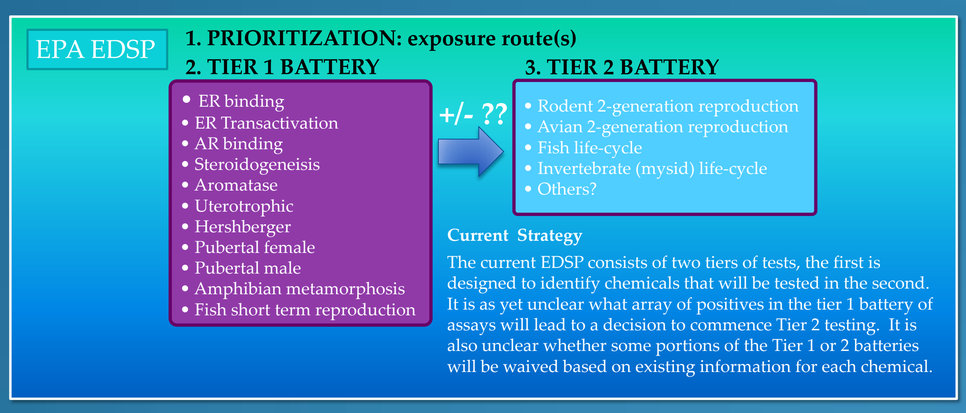


Table 1: EDSP Tier 1 Assays

	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	Uterine cytosol from rats	Estrogen agonists, antagonists	ER binding	effect ovary/uterus size, histology, male/female fertility
AR binding: OPPTS 890.1150	Prostate cytosol from rats	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	immature rats and mice: 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	rats, mice	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	rats	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	rats	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: rats or mice	12 months exposure: gross necropsy plus histopathology of adrenals, testes, epididymides, ovaries, uterus, thyroid, parathyroid.	R/CR
870.6200	90-day neurotoxicity	FOB: autonomic function, convulsions, tremors, abnormal motor movements, reactivity to general stimuli, general level of activity, posture and gait abnormalities, forelimb/hindlimb grip strength, foot splay, sensorimotor responses, body weight, neuropathology.	R/R
870.4200	Carcinogenicity	24 month exposure: gross necropsy, possible histopathology of adrenals, parathyroid, thyroid, prostate, testes, epididymides, seminal vesicle(s), uterus, ovaries, female mammary gland.	R/CR
870.3700	Prenatal developmental toxicity, rats and rabbit	Exposure throughout gestation: fetal deaths, resorption, 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. Histopathology 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 development, 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
850.2300	Avian reproduction	Eggs laid, percent fertilized, eggs not cracked, shell thickness, hatching, chick survival	R	R	R	R
850.1400 (OECD TG 210)	Fish early life stage (freshwater)	Exposure of eggs until hatching: cumulative mortality, # of healthy fish at end of test, time to start of hatching and end of hatching, # of larvae hatching each day, length and weight of surviving animals, # of deformed larvae, # of fish exhibiting abnormal behavior.	R	R	R	R
850.1500	Fish life cycle	Spawning, egg numbers, fertility, and fecundity.	CR	CR	CR	CR

*new in 2007

SUMMARY

We present here available relevant data for two EDSP Phase 1 chemicals using an integrated scheme to illustrate the data's relevance for fulfilling information requirements in place of new Tier 1 *in vivo* tests.

• Phase 1 chemicals, pesticides and HPV chemicals, have already been tested in Tier 2 tests, including multi-generation mammalian reproductive tests and in some cases assessment of effects on fish and bird reproduction and development.

• Other relevant human observational and epidemiological data and mechanistic *in vitro* and *in vivo* data are also available for most Phase 1 chemicals.

• For many of these chemicals, the mechanism of action and primary toxicological pathways have already been characterized.

•In subsequent phases the assessment of existing information related to endocrine-disrupting potential should take precedence over new testing and should be used to inform any subsequent testing.

•Chemicals can then be screened and prioritized in a consistent stepwise manner starting with high throughput *in vitro* and *in silico* characterization, completing an evidence-based analysis before moving on to additional testing.

For more detailed information on Phase I chemicals, see comments submitted to EPA from PETA and PCRM to Docket Number EPA–HQ–OPP–2009–0634 at www.regulationa.gov.

For more information, contact C. Willett at KateW@Peta.org