

QUESTIONS AND ANSWERS ABOUT U.S. ANIMAL TESTING OF ENDOCRINE DISRUPTORS

What does the term “endocrine disruption” mean?

Endocrine disruption refers to harmful effects on reproduction, development and/or health in general resulting from chemically-induced interactions with the body’s hormone system. Since the 1962 publication of *Silent Spring*, there has been concern that chemicals in the environment could be harmful to both wildlife populations and human health. The term “endocrine disrupter” was coined in 1991 by former World Wildlife Fund scientist Theo Colborn, who reported that some environmental chemicals can disrupt the body’s hormone system, and that effects of exposure during development can be permanent and severe.

What kinds of substances have been identified as suspected endocrine disruptors?

A range of synthetic as well as naturally occurring agents have been identified as potential endocrine disruptors, including synthetic hormones, pesticides, compounds used in the plastics industry and in consumer products, industrial by-products and pollutants, and even some naturally occurring substances in plants.

What is the U.S. doing to address endocrine disruptor concerns?

In 1996, the U.S. Congress enacted the Food Quality Protection Act (FQPA),¹ which introduced a number of significant amendments to both the Federal Food, Drug and Cosmetic Act and Safe Drinking Water Act, including a requirement that the Environmental Protection Agency (EPA) “develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect as [EPA] may designate.” Congress further directed that EPA “shall provide for the testing of all pesticide chemicals” and “may provide for the testing of any other substance that may have an effect that is cumulative to an effect of a pesticide chemical if [EPA] determines that a substantial population may be exposed to such substance.” In practical terms, this Congressional mandate will lead to substantial additional testing of nearly 5,000 pesticide active and other ingredients, and possibly thousands of other high production volume chemicals and environmental contaminants.

How has EPA responded to this mandate?

Responsibility for developing a “toolbox” of validated screens and tests for the emerging testing program has been delegated to the EPA’s Office of Science Coordination and Policy. EPA began by establishing a multi-stakeholder Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to provide the Agency with recommendations regarding:

- ▶▶ “Methods for chemical selection and priorities for screening”
- ▶▶ “A set of available, validated screening assays for early application”

¹ <http://www.fda.gov/opacom/laws/foodqual/fqpatoc.htm>

- ▶▶ “Ways to identify new and existing screening assays and mechanisms for their validation”
- ▶▶ “Processes and criteria for deciding when additional tests beyond screening would be needed and how to validate such tests.”

In its final report to EPA in September 1998,² EDSTAC called for the scope of the so-called Endocrine Disruptor Screening Program (EDSP)³ to be significantly expanded to encompass chemical interactions with two or more additional endocrine hormones (androgen and thyroid, as well as estrogen) in not only humans, but also in birds, fish, amphibians and invertebrates.

EDSTAC proposed a tiered testing framework to accommodate substances with differing amounts of available data:

- ▶▶ Tier 1 would consist of a battery of relatively rapid, inexpensive, and largely mechanistic screening assays, designed to detect chemical interactions with estrogen, androgen and thyroid hormones in mammalian, amphibian and fish species
- ▶▶ In Tier 2, substances which appear, on weight-of-evidence, to be endocrine-active may then be subject to multigenerational reproductive toxicity studies in up to five different taxonomic groups.

Using the EDSTAC report as a road map, EPA established a Standardization and Validation Task Force (which was later replaced by two similar committees⁴) to provide advice concerning the scientific and technical work necessary to validate the recommended screens and tests. EPA has also been working with other national governments through the Organization for Economic Cooperation and Development (OECD) to validate endocrine screens and tests of international interest.⁵

What tests are being developed to identify endocrine disrupting substances?

Screens to detect chemical interactions with estrogen, androgen and/or thyroid hormones include the following:

- ▶▶ Uterotrophic assay in rats or mice
- ▶▶ Hershberger assay in rats or mice
- ▶▶ Enhanced 28-day repeated dose toxicity study in rats
- ▶▶ Female and male pubertal assays in rats
- ▶▶ Intact male rat assay
- ▶▶ Amphibian metamorphosis assay
- ▶▶ Fish reproduction screen
- ▶▶ *In vitro* receptor-binding assays
- ▶▶ *In vitro* transcriptional/reporter gene activation assays
- ▶▶ *In vitro* steroidogenesis assays
- ▶▶ *In vitro* aromatase assays

Tests thought to provide more-definitive proof of harm to human and/or ecological health include the following:

- ▶▶ 2-generation reproduction study in rats
- ▶▶ 2-generation reproduction study in birds
- ▶▶ 2-generation reproduction study in frogs
- ▶▶ Fish full life-cycle test
- ▶▶ Mysid shrimp full life-cycle test

² <http://www.epa.gov/scipoly/ospendo/pubs/edspoverview/finalrpt.htm>

³ http://www.oecd.org/document/62/0,3343,en_2649_34377_2348606_1_1_1_1,00.html

⁴ <http://www.epa.gov/scipoly/ospendo/pubs/assayvalidation/edmvac.htm>

⁵ http://www.oecd.org/document/62/0,3343,en_2649_34377_2348606_1_1_1_1,00.html

How many animals are used in these screens and tests?

Tier 1 screening methods each consume between 20 and 80 animals⁶ (except for the *in vitro* methods, which do not consume any animals), while Tier 2 reproduction/lifecycle tests each consume between 2,600 and 5,500 animals per chemical tested. It is estimated that for every chemical evaluated in EPA's Tier 1 battery, in excess of 360 animals will be consumed. Thus, to screen each of the roughly 5,000 pesticide chemicals as the FQPA requires would consume approximately 1.8 million animals. Millions more could be consumed in follow-up Tier 2 testing of chemicals that yielded positive results in Tier 1.

Won't some of these tests already have been conducted on pesticides and chemicals?

Yes. For most pesticide active ingredients, reproductive toxicity studies on rats, birds and invertebrates, and lifecycle studies in fish, will already have been conducted, together with dozens of other tests to characterize the substance's potential toxicity.⁷ Thus, if a pesticide has already been deemed safe on the basis of studies that are substantially equivalent to EDSP Tier 2 "definitive" tests for endocrine disruption, it is highly doubtful that the results of Tier 1 screens (e.g., evidence of binding to the estrogen receptor) or duplicate Tier 2 tests would lead to any change in the way a pesticide or other substance is regulated.

Are animal tests accurate predictors of endocrine disrupting hazard to people?

Not necessarily.⁸ A 2003 white paper commissioned by EPA documented that chemical effects on the endocrine system can differ dramatically not only between animal species, but also between strains of the same species.⁹ This raises serious doubt concerning the human relevance of test results from rats or mice. Equally troubling are questions that have been raised as to whether OECD-run studies to validate one or more animal tests has complied with the internationally agreed standards.¹⁰

What are some practical alternatives to animal testing?

A number of the screens that have been developed for use under the EDSP are *in vitro* (i.e., cell lines and cellular components, such as isolated estrogen and androgen receptors), and could therefore replace or reduce animal use in some cases if conducted as part of a "tiered" testing strategy (i.e., if animal testing were undertaken only as a last resort). However, this is unlikely to be the case under the EPA's currently proposed "battery" approach, according to which all Tier 1 screens (animal and non-animal) would be performed concurrently, thereby precluding any potential reduction in animal use.

What is the status of testing under EPA's EDSP?

In June 2007, EPA published a draft list of 73 chemicals to be subject to initial screening under the EDSP, noting that "[t]he Tier 1 screening battery is expected to complete peer review and be ready for use in early 2008." Program implementation beyond this pilot phase (i.e., "the testing of all pesticide chemicals," per FQPA) will be left to the discretion of EPA's Office of Pesticide Programs.

⁶ This estimate does not include the 270 or more mother rats and "surplus" offspring produced in for each pubertal assay, nor the hundreds of eggs produced in the fish reproduction screens.

⁷ <http://www.hsus.org/web-files/PDF/ARI/pesticides.pdf>

⁸ http://www.hsus.org/animals_in_research/animal_testing/limitations-of-animal-methods.html

⁹ <http://www.stopanimaltests.com/pdf/strain.slides.pdf>

¹⁰ <http://ecvam.jrc.it/publication/Esac%20statement%20on%20uterotrophic%20assay2.doc>

What is the Humane Society doing to help animals used in testing?

The Humane Society of the United States and Humane Society Legislative Fund are actively working to end animal testing—permanently. We are working to promote greater reliance on available non-animal testing methods, and are actively supporting the vision of “twenty-first century toxicology” articulated by the U.S. National Research Council, which would see animal tests that are decades old, costly, slow and of dubious relevance to people replaced by ultra-modern, efficient and human-relevant non-animal methods.¹¹ We are calling for a “big biology” project to meet this challenge, akin to the Human Genome Project of the 1990s, and are forging an international, multi-stakeholder consortium make this landmark vision a reality as quickly as possible.



The Humane Society of the United States is the nation’s largest animal protection organization—backed by more than 10.5 million Americans. For over 50 years, HSUS has worked to reduce suffering and to create meaningful change for animals in laboratories through public education, scientific outreach, legislative advocacy, and strategic partnerships.

Online at HSUS.org/research

The Humane Society Legislative Fund is a social welfare organization incorporated as a separate lobbying affiliate of the HSUS. HSLF works to pass animal protection laws at the state and federal level, to educate the public about animal protection issues, and to support humane candidates for office.

Online at HSLF.org

¹¹ http://www.hsus.org/animals_in_research/animal_testing/hsus-projects/human_toxicology_initiative.html