Responses to Questions from the House of Representatives, Committee on Oversight and Government Reform, Chairman Henry Waxman, September 20, 2007

1. How can EPA speed up this process?

a. What is the minimum length of time manufacturers will need to test a chemical under Tier 1?

Of the assays being considered for the Tier 1 battery, the pubertal assays require the longest time to conduct, with an estimate of approximately 15 months. Assuming that this assay becomes part of the battery and that all assays in the battery can be conducted simultaneously, screening a chemical with the Tier 1 battery would require approximately 15 months to complete. This estimate includes pre-test activities (e.g., identification of appropriate testing labs, acclimation of test animals, development of QA/QC plans), in-life testing, and post-test processing (e.g., biochemical analyses, histopathology, data analyses, report writing and preparation of data submission to manufacturer and EPA). However, this estimate does not take into account limitations on laboratory capacity. Past experience with data requests and submissions to EPA indicate that 15 months from manufacturers' commitment to generate data until EPA's receipt of data would be a very ambitious schedule. We anticipate that for the first 73 chemicals additional time will be required to allow for constraints on both testing capacity and knowledge of how assays need to be conducted.

b. What is the minimum length of time manufacturers will need to test a chemical under Tier 2?

Considerations similar to those mentioned in answer 1a for the Tier 1 battery need to be factored in to the timelines for Tier 2 testing. Tier 2 tests, which cover 2 generations and a substantially larger number of animals, take much longer to complete – usually two years. Therefore, the Agency anticipates that it would be up to 3 years from the time at which the tests are requested before the Agency receives any Tier 2 data. Historically, EPA has set a deadline of 48 months to submit the results of a 2 generation rat reproductive toxicity study.

c. How many chemicals can be tested simultaneously?

At this time the Agency does not have a basis to estimate available testing capacity, and therefore the number of chemicals that can be tested simultaneously. Once Tier 1 screening for the initial group of chemicals is underway, the ability to forecast testing throughput should improve.

It should be noted that available laboratory testing capacity may affect the testing capacity. While insufficient laboratory capacity may initially limit the number of chemicals that can be tested simultaneously, EPA anticipates that the market will adjust

to the growing need for laboratory capacity once testing orders are issued, increasing the number of chemicals that can be tested simultaneously.

d. Can the time for testing be shortened?

As indicated in the response to Question 1, the pubertal assay will be the assay that likely will determine how quickly the battery can be completed, and this assay is expected to require approximately 15 months to complete. However, shorter timeframes are anticipated for other assays (e.g., 6 months for *in vitro* assays, 12 months for amphibian and fish screens). Thus, EPA may begin receiving data and can initiate its review of data earlier than 15 months after issuance of testing orders.

The Tier 1 battery being validated by EPA is based on recommendations from a Federal Advisory Committee, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). The EDSTAC concluded that the Tier 1 assay battery they envisioned would require significant resources, animals and time to test a large number of chemicals. Consequently, EDSTAC also recommended that the Agency develop predictive computer models (e.g., quantitative structure activity relationships models) and *in vitro*-based high throughput screening assays to help prioritize chemicals for *in vivo* Tier 1 assays to ensure limited testing capacity be directed to those compounds with the greatest likelihood of causing endocrine disruption. Initial, exploratory efforts by the Agency in the 2000 timeframe, while promising, indicated that significant research and development efforts would be required to establish these technologies. Consequently, the Agency focused the majority of its resources on completing the development and validation of the current Tier 1 assay battery to initiate the screening program. The Agency has continued research and development efforts in modeling and high throughput screening, with some success, and it is possible these technologies could be brought to bear for specific endpoints and chemical inventories to help prioritize *in vivo* screening and testing in the future.

2. When will EPA publish subsequent draft and final lists of chemicals to be tested?

It is anticipated that subsequent testing priorities will be based on Agency data needs (e.g., part of the Office of Pesticide Programs' pesticide Registration Review process, or Office of Water's development of the Contaminant Candidate List).

The current draft list of 73 chemicals will be finalized early next year in advance of issuing testing orders. As noted above, EPA expects to begin receiving data on these chemicals within a year and to have substantially all of the results from required Tier 1 testing about two years after manufacturers commit to producing the data. This body of data will provide an invaluable resource in helping EPA to understand how to optimize the Tier 1 battery to screen active and inert ingredients for potential effects on the endocrine system. As recommended by the FIFRA Scientific Advisory Panel (SAP), EPA plans to evaluate the data not only to assess individual chemicals' effects on the endocrine system but also to determine whether to make adjustments to the testing battery. The Agency would seek external peer review (e.g., through the SAP) on any

revisions to the Tier 1 battery. After we have an optimized battery, we anticipate making endocrine disruptor screening a routine part of the registration review process. The current schedule through 2010 is at http://www.epa.gov/oppsrrd1/registration_review/schedule.htm. Of course, EPA has discretionary authority to issue, at any time, additional testing orders requiring manufacturers to conduct Tier 1 assays.

For chemicals other than pesticide active ingredients, as new technologies for screening large numbers of chemicals emerge (e.g., http://www.epa.gov/comptox/toxcast), testing priorities may also be based on hazard. The number of inerts and drinking water contaminants that can be screened annually will depend on what is learned from the first set of nine pesticide and High Production Volume inerts included in the proposed set of 73 chemicals, including lab capacity and resource availability.

3. Will the agency provide for concurrent testing of chemicals or does it intend to complete the process for the first set of chemicals prior to beginning the next set?

As explained above, the Agency would like to assess the performance of the Tier 1 battery on the first set of 73 chemicals before beginning to routinely issue additional testing orders. The Agency acknowledges that this approach would delay issuing a second round of Tier 1 test orders. However, testing of specific additional chemicals concurrent with the testing of the first 73 may be required should the need arise.

4. How many chemicals does EPA anticipate it will include on subsequent draft lists?

As indicated above in the answer to question 2, the number of chemicals will be driven by the Agency's data needs. For example, candidate chemicals will include the pesticide active ingredients for which registration review will be initiated. In addition, a number of pesticide inert ingredients and drinking water contaminants could also be screened.

5. Will EPA commit to identifying a minimum number of chemicals to be tested each year? How many?

As indicated in the previous responses, once the Tier 1 battery becomes a routine component of the registration review process, EPA will evaluate all pesticide active ingredients that are initiating pesticide registration review, (i.e., approximately 70 pesticide cases per year) for their potential to affect the endocrine system. In addition, a number of pesticide inert ingredients and drinking water contaminants could also be screened. To ensure that no unnecessary testing is required, EPA will review any existing test data before initiating further testing.

a. At that rate, when will all of the pesticide chemicals be screened as the statute requires?

Assuming that 70 pesticide active ingredient cases were opened each year under the registration review program, it would take approximately 15 years to complete the process of requiring data and completing the screening of all of the 680 pesticide cases comprising about 1,080 active ingredients. It would take considerably longer to screen the approximately 2,775 inert pesticide chemicals with the existing technologies.

As noted in the response to question # 2, for several years EPA has been investing substantial resources in the development and validation of high throughput assays and predictive computer models for endpoints involving the endocrine system. If this research is fruitful, it may be possible both to set priorities for further testing and to screen large numbers of chemicals in a shorter period of time in the future.

b. At that rate, when will all the other chemicals of potential concern be screened under the SDWA authority?

Using existing technology, it would take decades to screen all possible chemicals under the SDWA authority. However, many of the contaminants found in sources of drinking water are also pesticide active ingredients and inerts and would be tested under 408(p) authority. Also, testing of other chemicals under SDWA authority can occur concurrently with the testing of pesticide chemicals. As with pesticide chemicals the Agency expects that, when available, fully validated high throughput approaches will greatly accelerate this process. Additionally, under the SDWA the Drinking Water program utilizes all available information to evaluate chemicals that may be found in sources of drinking water and may be of potential concern.

6. When will the Tier 1 testing battery be finalized?

Peer review of the candidate Tier 1 assays is currently underway. EPA will review the results of these peer reviews and propose the Tier 1 battery for review by the FIFRA Scientific Advisory Panel in early 2008.

The current peer review schedule for the assays under consideration for Tier 1 is shown below. Peer review is the final stage in the validation process and entails independent scientific review of the standardized assay protocols.

Tier 1:

▪ Uterotrophic	Complete
▪ Hershberger	Complete
▪ Adult Male	In Review
▪ Female Pubertal	In Review
▪ Male Pubertal	In Review
▪ AR Binding	2007-Q4
▪ Aromatase	2007-Q4
▪ Amphibian Metamorphosis	2007-Q4
▪ Fish Screen	2007-Q4
▪ Steroidogenesis	2008-Q1

- ER Binding 2008-Q2

7. When will EPA finalize the testing procedures for the Tier 1 tests?

EPA expects to issue the proposed testing procedures in a Federal Register notice in the next month. After a 90-day comment period, EPA will review and finalize the procedures. It is anticipated that the final testing procedures will be issued in a Federal Register notice in early 2008.

8. When will Tier 1 testing orders be issued to PMPs for the first list of 73 chemicals?

EPA anticipates that Tier 1 test orders for the first 73 chemicals will be issued by mid-2008.

9. How much time will PMPs be given to execute these tests?

As indicated in the response to Question 1a, we expect that up to 15 months will be needed before all data for the entire Tier 1 battery will be submitted to the Agency for review. Some data may be submitted sooner. If there are technical difficulties (e.g., incompatibility of chemical with specific assay) or lab capacity issues, other data may be submitted later.

10. When EPA receives testing results from PMPs, how long will it take the agency to determine which chemicals must be tested using Tier 2 tests?

Once received, test data for the Tier 1 battery will be reviewed by EPA, and a weight-of-evidence determination will be made to decide whether Tier 2 testing will be required for the chemical. This process will take up to 1 year.

11. When will the Tier 2 battery be finalized?

We anticipate the five Tier 2 tests will be finalized by no later than 2010. It is important to note that these tests will not be used as a battery and therefore they can be employed as they are validated for use.

The current peer review schedule for the assays under consideration for Tier 2 is shown below. Peer review is the final stage in the validation process and entails independent scientific review of the standardized assay protocols.

Tier 2:

- Mammalian 2-generation Complete
- Avian 2-generation 2009/2010
- Amphibian Growth/Reproduction 2009/2010
- Fish 2-generation 2009/2010
- Mysid 2-generation 2009/2010

12. When will EPA finalize the testing procedures for the Tier 2 tests?

EPA expects that the forthcoming draft testing procedures will be applicable to both Tier 1 and Tier 2 testing. However, a separate Information Collection Request (ICR) will be required to address the information collection activities associated with Tier 2 testing. Validation of the Tier 2 assays should be complete in 2010, and the ICR for Tier 2 testing will be finalized in a timeframe that will ensure no delay in Tier 2 testing that is deemed appropriate based on the results of Tier 1 screening. Also, if EPA determines, based on experience gained from Tier 1 testing, that modifications to the testing procedures are necessary, these revisions will be made before validation of the Tier 2 testing is complete.

13. When will Tier 2 testing orders be issued to PMPs, for those chemicals that fail the Tier 1 screens?

Once the ICR for Tier 2 testing is in place and the Tier 2 tests are validated (i.e., 2010), orders can be issued for chemicals that are identified in the Tier 1 screens as having the potential to interact with the endocrine system. Since the mammalian two-generation test is already considered validated, orders for this test could be issued immediately following Tier 1 data review as long as the ICR is in place.

14. How much time will PMPs be given to execute these tests?

It is anticipated that Tier 2 testing will take approximately 18 to 24 months, which includes the time animals are exposed to a compound (i.e., the in-life portion may require up to 9 months for some tests) as well as sample and data analyses. Historically, EPA has set a deadline of 48 months to submit the results of a 2 generation rat reproductive toxicity study.

15. When EPA receives testing results from pesticide manufacturers, how long will it take the agency to weigh the results, and if necessary, issue a new regulation for a chemical of concern?

EPA does not necessarily need to issue a regulation to address any risks of concern indicated by the data. For example, pesticide regulation under FIFRA does not typically proceed by rulemaking, but through amendments to the license through adjudication. Depending on the strength of the concern, that process can take effect immediately, in the form of an emergency suspension to the license, or can take several years if the pesticide registrant contests the Agency's regulatory conclusions.

16. What tests will be included in the Tier 1 battery? What effects will these tests screen for?

Subsequent to passage of the Food Quality Protection Act in 1996, EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), a committee of scientists and stakeholders that was charged by the EPA to provide

recommendations on how to implement its Endocrine Disruptor Screening Program (EDSP). EDSTAC recommended candidate assays for the Tier 1 battery as shown in the following Tables. These are the assays that EPA is in the process of validating individually (including peer review). The composition of the battery will be determined, based on advice from EPA's Scientific Advisory Panel, after peer review of the individual assays is complete.

Table 1. Tier-1 *in vitro* and *in vivo* screening assays recommended by the EDSTAC.

Assays	Reasons for consideration
Estrogen receptor (ER) binding or transcriptional activation	A sensitive *in vitro* assay to detect chemicals that may affect the endocrine system by binding to the ER.
Androgen receptor (AR) binding or transcriptional activation	A sensitive *in vitro* assay to detect chemicals that may affect the endocrine system by binding to the AR.
In vitro steroidogenesis	A sensitive *in vitro* assay to detect chemicals that interfere with the synthesis of the sex steroid hormones.
Uterotrophic (rat)	An *in vivo* assay to detect estrogenic chemicals. It offers the advantage over the binding assay of incorporating absorption, distribution, metabolism, and excretion (ADME)
Hershberger (rat)	An *in vivo* assay to detect androgenic and anti-androgenic chemicals. It offers the advantage over the binding assay of incorporating ADME and differentiating between AR agonists and antagonists.
Pubertal female (rat)	An assay to detect chemicals that act on estrogen or through the hypothalamus-pituitary-gonadal (HPG) axis that controls the estrogen and androgen hormone systems. It is also enhanced to detect chemicals that interfere with the thyroid system.
Frog metamorphosis	A sensitive assay for detection of chemicals that interfere with the thyroid hormone system.
Fish screen	An *in vivo* assay for detection of chemicals that interfere with the HPG axis. Fish are the furthest removed from mammalians among vertebrates both from the standpoint of evolution—their receptors and metabolism are different from mammals—and exposure/habitat, since they would be subject to exposure through the gills, whole body, and diet. Thus, the fish assay would augment information found in the mammalian assays and would be more relevant than the mammalian assays in triggering concerns for fish and other vertebrates.

Table 2. Alternative *in vitro* and *in vivo* assays recommended for the Tier-1 Screening Battery by the EDSTAC.

Assays	Reasons for consideration
In vitro aromatase	The aromatase assay detects chemicals that inhibit the enzyme that converts androgens to estrogen and would be needed if either of the two following assays using males were substituted for the female pubertal assays. The male is not believed to be as sensitive to alterations in aromatase as the female and would not therefore be sufficient to detect interference with aromatase in the screening battery.
Pubertal male (rat)	The assay detects chemicals that act on androgen or through the HPG axis that controls the estrogen and androgen hormone systems. It is also enhanced to detect chemicals that interfere with the thyroid system. This assay could in part substitute for the female pubertal assay.
Adult male (rat)	The assay is also designed to detect chemicals that act on androgen or through the HPG axis that controls the estrogen and androgen hormone systems. It is also enhanced to detect chemicals that interfere with the thyroid system. This assay could in part substitute for the female pubertal assay.

17. Staff mentioned that a test for prenatal effects will not be included in either the Tier 1 or Tier 2 batteries. Why is this the case, when endocrine disrupting chemicals can have such profound effects on developing fetuses?

In utero (prenatal) mammalian exposures will not be included in the Tier 1 battery, but this life stage is covered in the existing rat Tier 2 multigenerational test. Although not part of the EDSP the rabbit developmental test and the rat developmental neurotoxicity test, which are routinely required to support pesticide registrations, also cover this life stage. EPA is currently working with our international partners in OECD to develop a shorter protocol for the two-generation rat study that can substantially reduce the amount of time the test would take to complete - approximately 6 months to a year less than the current two-generation mammalian reproductive effects test protocol. Also, the amphibian metamorphosis assay is conducted with larval frogs (tadpoles) which are considered to be developmentally analogous to a mammalian fetus.

EPA also acknowledged that the developmental period is an important life stage to study in any screening program. EPA also indicated that the program has been working with the scientific community to see if a meaningful *in utero* mammalian Tier 1 screen could be developed (i.e., whether it is technically feasible). The EDSP recently convened a Federal Advisory Committee to address this issue (see htttp://www.epa.gov/scipoly/sap/meetings/index.htm#february). At this meeting the independent scientists, as well as the EPA scientists, acknowledged the desirability for such a test, but were unable to recommend any possible candidates. EPA will continue to explore possible options as new assays are proposed and developed.

18. We understand that there has been some concern over the rat strain that EPA will require manufacturers to use in the Tier 1 tests – specifically, that EPA may require a strain that is less sensitive to some endocrine disrupting effects. Is EPA aware of this concern? Has EPA made a decision on what strain to use? If so, what is it? If not, what strains are the agency considering? Will EPA commit to not selecting a rat strain that may not be sufficiently sensitive to endocrine disrupting effects?

EPA is aware of the concerns that the strain of rat used may affect the ability to detect a response in some endocrine assays. EPA prepared a white paper on the issue of rat-strain effects on pubertal assay endpoints (see http://www.epa.gov/scipoly/oscpendo/pubs/edmvs/strainswhitepaper072503.pdf) The white paper and an expert reviewer's comments were reviewed by a Federal Advisory Committee in 2003. Based on this review, EPA concluded that although it appears that some strains of rats may be differentially sensitive to endocrine effects at specific endpoints for specific chemicals, it is not possible at this time to determine which strain will be the most susceptible across all (or most) endpoints in a single assay for all chemicals. That is, a "most sensitive" strain or set of strains has not been identified, and considerable research appears to be necessary to determine if an optimal strain or set of strains can be identified. EPA felt that such research, if undertaken, would delay the initiation of testing substantially.

The basis for selecting the Sprague Dawley (SD) rat over other rat strains is that it has often been the animal model of choice for determining general toxicological and, to a

lesser extent, endocrinological effects. More recently, SD rats have been used to examine specific endocrine-mediated effects of natural and synthetic compounds on reproduction and thyroid function in intact rodent models. Many laboratories use SD rats for multi-generation studies, including the two-generation reproduction toxicity test currently proposed for Tier 2, and therefore, this model will allow for an examination of reproducibility of endpoints common to Tiers 1 and 2 in the same strain of rats. Furthermore, relatively large historical data bases are available for reference. While the EPA recognizes there are reasons to believe that this strain might be less sensitive to certain endocrine endpoints, the data currently available appear to show that it is no worse (or better) than other strains for screening for endocrine activity.

EPA prefers to standardize on the Sprague-Dawley rat, but will allow use of Wistar rats in the pubertal assays. This is based on data showing comparability of results between Sprague-Dawley and Wistar rats when the same chemical has been tested in both strains.

19. What tests are in the Tier 2 battery? What effects will these tests screen for?

Tier 2 is expected to include the multi-generation tests in mammals, fish, birds, amphibians (partial life-cycle), and invertebrates. They will determine whether chemicals affect fertility, sexual differentiation, development, and growth. Again, as for Tier 1, the composition of Tier 2 depends on whether the tests can be validated for this use.

20. How does the agency plan to weigh conflicting evidence put forward by PMPs? What if the same pesticide passes a Tier 1 test undertaken by one PMP, but fails the same test undertaking by another PMP?

Consistent with the statutory goal of minimizing duplicative testing, EPA will establish procedures that encourage recipients of test orders to work collaboratively to generate a single set of data on each chemical subject to testing orders. Thus, we do not expect this situation to arise often. Should multiple PMPs submit data on the same chemical for the same assay, the Agency's interpretation would depend on the overall quality of the data set. All things being equal, the Agency would take a conservative (protective) approach in interpreting data. Conflicting data or equivocal data would likely result in a decision to request Tier 2 testing or other appropriate testing.

21. How does the agency plan to weigh conflicting evidence put forward by PMPs in Tier 2 tests?

As explained in the response to question # 20, we do not foresee this circumstance will arise with any frequency. But, as with all other agency evaluations of data sets, the Agency would conduct a case-by-case, weight of the evidence evaluation of all available evidence, and that overall represents a conservative (protective) approach in interpreting data. Weight of evidence evaluation is a collective evaluation of all pertinent information so that the full impact of biological plausibility and coherence is adequately considered. Judgment on the weight of evidence involves consideration of the quality and adequacy

of data and consistency of responses induced by the stressor. Generally, no single study, whether positive or negative, drives the overall weight-of-evidence judgment.

22. How does the agency plan to weigh conflicting evidence gathered in the two rounds of testing?

Tier 2 data would be used to determine if effects occur and at what level for regulatory purposes; by contrast, Tier 1 is designed as a screen to identify which chemicals have the potential to interact with the endocrine system, and consequently warrant further testing. Thus, the data from Tier 2 testing are intended to resolve any questions raised by the results of the Tier 1 screens, and would ultimately outweigh results from the Tier 1 screens.

23. We understand that EPA selected the chemicals included on the first draft list based on potential for exposure. Will EPA use a similar methodology to select chemicals to be included on subsequent lists?

As EPA indicated in the Sept 2005 FR notice, the Agency will likely modify its priority setting approach. As discussed in the answer to question 2, it is anticipated that subsequent testing priorities will be based on agency data needs (e.g., part of the Office of Pesticide Programs' Pesticide Registration Review process, or Office of Water's development of the Contaminant Candidate List). Also, EPA is in the process of developing additional tools that may assist in the priority setting process, as mentioned in response to Question 2.

24. How will EPA consider substances other than pesticides for inclusion on future draft lists? In particular, what are the agency's plans for including chemical mixtures and persistent and bioaccumulative chemicals present in drinking water, waste water and commonly used consumer products? Will EPA commit to including a minimum number of such chemicals in each list?

At this time, the Agency cannot commit to any minimum number of such chemicals on future lists. Consistent with the capabilities of existing scientific tools and available resources, it is the intent of the Agency that such chemicals and mixtures be screened for possible endocrine effects. As indicated in the response to Question 2, selection of chemicals on subsequent lists will be based on Agency needs and could be influenced by newer high throughput approaches, as a means to prioritize chemicals for screening.

25. EPA has included on its initial draft list for Tier 1 testing a number of chemicals – such as atrazine—that are known endocrine disruptors. Why is EPA wasting valuable agency resources by subjecting such chemicals to Tier 1 tests, when they could be immediately moved to the more intensive Tier 2 testing program?

Based on previous testing, EPA has some endocrine related data on some of the chemicals on the draft list. However, none of the chemicals on the draft list have been tested using all of the newly validated EDSP assays. The purpose of Tier 1 screening is

to integrate information for a given chemical across all the assays to develop a weigh of evidence analysis to determine if the compound has the potential to disrupt the endocrine system. Also, EPA expects that the mechanistic data obtained from Tier 1 would be useful for interpreting Tier 2 data and may be useful in identifying chemicals that may have an effect that is cumulative to an effect of a pesticide chemical as stipulated in FFDCA 408(p). Therefore, the Agency believes it is valuable to test chemicals, such as atrazine, to determine their performance in the entire Tier 1 battery. The Agency does not view this testing as a waste of Agency resources (industry is required to conduct the tests, not the Agency). For food-use active ingredients, such as atrazine, mammalian 2 generation data (one of the Tier 2 tests) already exist. Since other Tier 2 tests will not be validated until the 2010 time frame, testing such chemicals in Tier 1 will not result in any significant delays.

26. Why has EPA failed to set up a nomination process by which members of the public may submit suggestions for chemicals to be considered for draft listing? Does the agency plan to initiate such a program?

EPA believes that nominations from the public are important because they provide a mechanism to identify chemicals which may result in high exposures in local communities but which may not otherwise receive national attention. However, EPA has previously published in the Federal Register that the initial list of chemicals would be based on exposure considerations alone, and that it would defer consideration of nominations from the public until subsequent testing lists. The rationale behind this decision was to keep this initial effort administratively simpler and thus ensure that a set of test results can be obtained in a timely manner for a mid-course evaluation of the EDSP Tier 1 screening battery. The Agency will consider developing a nomination process that would work in concert with other priority setting mechanisms in the future.

27. We understand that the public is unable to notify EPA when it can demonstrate that a chemical is a known endocrine disruptor and should be moved immediately to the second round of testing. This process would save valuable agency resources and grant the public a right already extended to pesticide manufacturers. Does EPA plan to initiate such a process?

Although EPA has not established a specific process by which the public can petition the Agency to take action with respect to individual chemicals, members of the public currently may provide any information they have, and petition the Agency to expedite the testing of those substances. No specific process or authorization is necessary to allow the public to do so.