U.S. Environmental Protection Agency
High Production Volume Chemicals Challenge Program: Accounting for the Animals

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Introduction

Announced in October 1998, the U.S. Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemicals Challenge Program "challenged" industry to make baseline health and environmental effects data publicly available on nearly 2,800 HPV chemicals. The data required were based on the Organization for Economic Cooperation and Development's (OECD) HPV Screening Information Data Set (SIDS) Program. Program participants sponsored HPV chemicals that they produced or imported, committing to provide the required data to EPA. Participants prepared test plans for each chemical or chemical category in which they evaluated the adequacy of existing data to meet the SIDS data requirements and addressed any data gaps. EPA posted these test plans on its program web site to provide the public with an opportunity for review and comment. Although participants were directed not to initiate proposed testing until these comments were reviewed, testing began prior to the close of the comment period in a number of cases.

Most of the required health effects endpoints were animal-based. These included acute, repeat dose, developmental, reproduction and possibly genetic toxicity (Table 1). In addition, one of the required environmental effects endpoints was acute toxicity to fish. For each chemical subject to a full battery of tests, approximately 740 animals were required. If these tests were conducted for every chemical in the program, approximately two million animals would be killed. Upon learning of the program, PETA successfully campaigned to have at least minimal measures to reduce animal use incorporated into the program and for the exclusion of terrestrial toxicity testing on birds. PETA and the Physicians Committee for Responsible Medicine (PCRM) reviewed all 424 test plans and submitted official comments on 98% of them.

Data collection and development for the HPV Chemicals Challenge Program is nearing its end. Approximately 1,400 chemicals are included in the original and revised test plans and EPA comments reviewed here. For cases in which participants have not submitted revised test plans, EPA comments are assumed to represent current testing proposals. We estimate that 158,000 animals were required for tests proposed in latest test plan revisions (Figure 1). In this retrospective analysis, we account for these animals and for reductions in animal use. We also evaluate industry's compliance with, and EPA's administration of, program requirements for the use of animals.

Animal Use

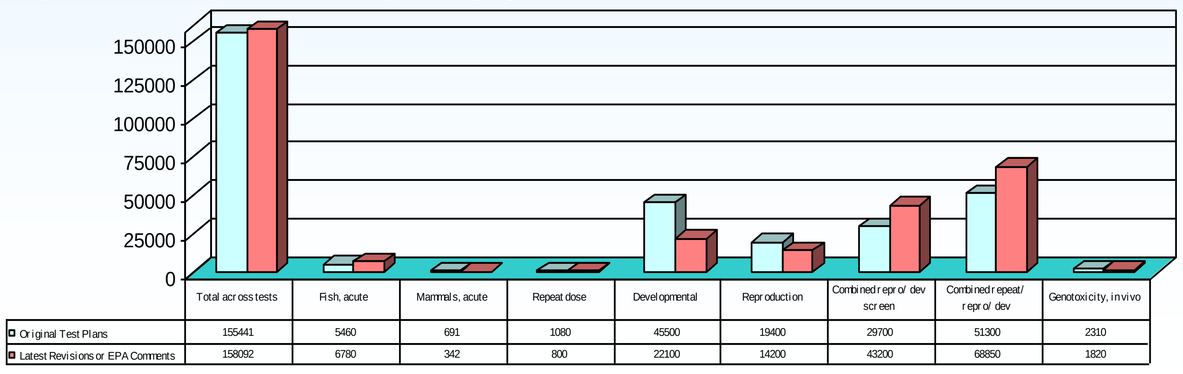
Table 1: Numbers of animals required for commonly proposed tests.

ENDPOINT	OECD TG	CALCULATION	ANIMALS USED
Fish, Acute	203	10 fish per group x (6 groups)	60 fish
Acute Oral	425	5 mammals for limit test + 1 mammal for each subsequent step	7 mammals
Repeated Dose 28-day Oral	407	10 mammals/group x 4 groups	40 mammals
Repeated Dose 90-day Oral	408	20 mammals/group x 4 groups	80 mammals
Prenatal Developmental	414	F0: 25 mammals/group x 4 groups + F1: 100 litters x 12 mammals/litter	1,300 mammals
1-Generation Reproduction	415	F0: 25 mammals/group x 4 groups + F1: 100 litters x 12 mammals/litter	1,300 mammals
Reproduction/Developmental Screening	421	F0: 24 mammals/group x 4 groups + F1: 48 litters x 12 mammals/litter	675 mammals
Repeated Dose with Reproduction/ Developmental Screening	422	F0: 24 mammals/group x 4 groups + F1: 48 litters x 12 mammals/litter	675 mammals
Mammalian Erythrocyte Micronucleus	474	14 mammals/group x 5 groups	70 mammals

Table 1 lists the animal tests most commonly proposed in the HPV Chemicals Challenge Program. Approximate numbers of animals used is calculated based on published OECD test guidelines (TG)[1]. At a minimum, data from OECD TG 425, 422 and 203 were required to address all health and environmental effects endpoints.

Figure 1 compares the numbers of animals required for tests proposed in original test plans and in latest test plan revisions. We estimate that the total number of animals required for all tests rose slightly after revisions and comments. The number of fish required for acute toxicity tests rose by 24%. The number of mammals required for *in vivo* genotoxicity tests fell by 21%. The number of mammals required for separate repeat dose, reproduction and developmental toxicity tests fell by 41%, but the number required for combined protocols rose by 38%.

Figure 1: Numbers of animals killed throughout the program.



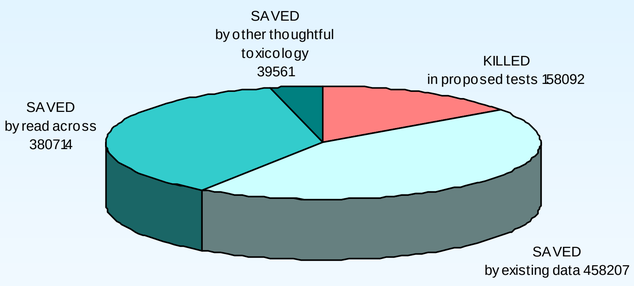
Conclusions

The original and revised test plans and EPA comments reviewed here address nearly 1,400 chemicals. If a full battery of tests were conducted for each of these chemicals, 1,034,000 animals would be used. Instead, we estimate that 158,000 animals were used for tests proposed in latest test plan revisions with a minimum of 73,000 animals already killed in tests conducted to date (Figure 5).

Compared to the number of animals required for all tests proposed in original test plans, the number of animals required after revisions and comments rose slightly. The number of mammals required for *in vivo* genotoxicity tests fell by 21% as a result of PETA and PCRM (and EPA in some cases) calling for these tests to be replaced with *in vitro* methods. While the number of animals required for separate repeat dose, reproduction and developmental toxicity tests fell by 41% as a result of these tests being replaced by combined protocols, the number of animals required for combined protocols rose by 38% – higher than expected simply as a result of replacing separate tests. The number of fish required for acute toxicity tests rose by 24%. These increases resulted from EPA claiming that uses of existing data, ECOSAR estimation and other thoughtful toxicology measures were unjustified in original test plans.

Altogether, the use of thoughtful toxicology measures saved approximately 880,000 animals or 85% of the more than one million who might otherwise have been killed. The use of existing data accounted for more than half of animals saved – approximately 460,000 in latest test plan revisions. Nevertheless, PETA, PCRM and EPA reported additional existing data for 60 original test plans – 28% of those in which animal tests were proposed – indicating that program participants often failed to adequately research the known toxicities of sponsored chemicals. "Read across" from data for related chemicals accounted for 381,000 animals saved in latest test plan revisions. PETA and PCRM argued that read across was underutilized, while EPA claimed that category justifications were inadequate in 43% of original category test plans, clearly indicating that EPA failed to play an active role in categorizing chemicals.

Figure 4: Numbers of animals saved and killed in proportion to the total number potentially required.



We estimate that other thoughtful toxicology measures accounted for the remaining 40,000 animals saved in latest test plan revisions – 12,000 fewer than in original test plans. PETA and PCRM called for these thoughtful toxicology measures to be applied more broadly, doubling the number of animals saved, while EPA more often claimed that their use was unjustified. In reviewing test plans, PETA and PCRM documented numerous failures to apply even the most basic thoughtful toxicology. For example, the American Petroleum Institute conducted repeat dose, reproduction and developmental toxicity tests on the petroleum gases ethane, butane, propane, and isobutane. Existing data generally show no-observed-effect- levels for these gases that were higher than their explosive levels! A number of sponsors conducted additional animal testing on naturally occurring substances that are already regulated as food additives or fish toxicity testing on insoluble materials.

While thoughtful toxicology measures dramatically reduced the number of animals required, many more opportunities to further reduce this number were ignored. Program participants failed to adequately research existing data, fully categorize related chemicals or apply basic thoughtful toxicology measures, and EPA failed to require participants to adhere to these measures. In addition, when participants did attempt to apply these measures, EPA frequently claimed that their justifications were inadequate.

We are hopeful that future chemical screening programs will rely heavily on the non-animal testing strategies put forth by the National Academy of Sciences in 2007 and in the 2009 EPA Strategic Plan.[7,8] Where animal testing is still required by regulatory agencies, this review of the U.S. EPA's HPV Chemicals Challenge Program provides quantitative evidence that careful consideration of human experience and exposure, gathering and consideration of existing data, categorization of chemicals, and use of validated replacement and reduction measures as they become available will greatly reduce animal suffering.

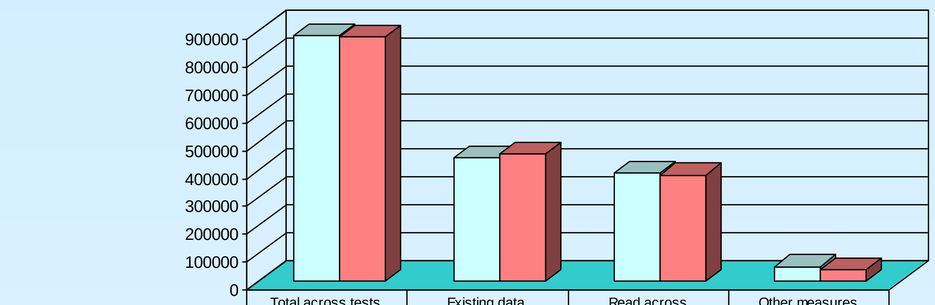
Reducing Animal Use

In an October 1999 letter[2], EPA directed program participants to "conduct a thoughtful, qualitative analysis rather than use a rote checklist approach," that they "may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested" and that they "should further consider whether any additional information obtained would be useful or relevant." EPA then listed specific thoughtful toxicology measures – incorporated into the program following pressure from PETA – that participants were to follow to reduce animal use.

Existing Data
EPA directed program participants to "maximize the use of existing and scientifically adequate data to minimize further testing." After accounting for tests proposed and conducted as well as for the other reduction measures detailed below, we estimate that 440,000 animals were saved as a result of the use of existing data in original test plans (Figure 2). PETA and PCRM (and EPA in some cases) reported additional existing data for 60 test plans – 28% of those in which animal tests were proposed, saving 16,000 additional animals in latest test plans revisions.

Categories and structure activity relationships (SAR)
EPA also directed program participants to "maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships." Grouping related chemicals into categories based on SAR reduces the number of animals required by allowing data on one chemical to be "read across" to other category members and also provides a contextual basis for evaluating toxicity. 120 original test plans were for categories of related chemicals, accounting for 77% of chemicals included in test plans submitted to date. We estimate that 390,000 animals were saved as a result of read across in these test plans. While PETA and PCRM called for the use of read across to be increased by 27% in test plans in which animal tests were proposed, EPA claimed that category justifications were inadequate in 43% of original category test plans. Participants generally responded by strengthening category justifications, resulting in a smaller increase in the number of animals used than if they had withdrawn the categories.

Figure 2: Numbers of animals saved throughout the program.



Other thoughtful toxicology measures
EPA listed several other thoughtful toxicology in its October 1999 letter, in a December 2000 Federal Register Notice entitled *Data Collection and Development on High Production Volume (HPV) Chemicals*[3] and in its *Guidance for Meeting the SIDS Requirements*:[4]

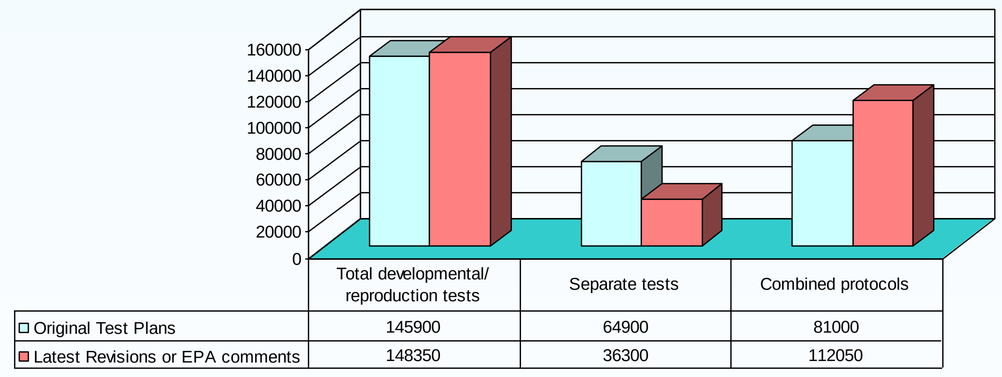
- not conducting repeat dose or reproductive toxicity test on closed-system intermediates;
- not conducting fish acute toxicity tests on hydrophobic chemicals (see *Fish Acute Toxicity*); and
- using effects on reproductive organs observed in repeat dose tests as the reproduction toxicity endpoint if a developmental study is also available.

In reviewing submitted test plans, PETA and PCRM also emphasized not conducting animal tests on highly corrosive or reactive chemicals or explosive gases and using data on the hydrolysis products of chemicals and major constituents of complex mixtures where applicable.[5]

Accounting for each of these specific measures, we estimate that 52,000 animals were saved as a result in original test plans (Figure 2). In contrast, 22 test plans in which animal tests were proposed were simply checklists providing no supporting rationale for the proposed tests. PETA and PCRM called for these thoughtful toxicology measures to be applied in at least 90 more cases, saving an additional estimated 40,000 animals. However, EPA frequently claimed that the use of these thoughtful toxicology measures was unjustified, resulting in an additional 12,000 animals being required in latest test plan revisions (Figure 2).

Combined test protocols
EPA "strongly recommended" the use of combined protocols, OECD TG 421 and 422, for repeat dose, developmental and reproduction toxicity. Each of these require roughly half the number of animals as either of the separate developmental or reproduction toxicity tests, OECD TG 414 and 415 (Table 1). Conducting an OECD TG 421 or 422 test in place of both OECD TG 414 and 415 tests (and possibly a repeat dose test as well) therefore reduces the number of animals required by approximately 2000. We estimate that 153,000 animals were saved as a result of combined protocols being proposed in original test plans compared to 240,000 who would have been required if separate tests had been proposed instead. 41% fewer animals were required for separate tests in latest test plan revisions as a result of these tests being replaced with combined protocols. However, 38% more animals were in fact required for combined protocols in latest test plan revisions because EPA also claimed that the use of existing data and other thoughtful toxicology measures were inadequate to satisfy the requirements for these endpoints in original test plans, resulting in new combined protocols being proposed.

Figure 3: Numbers of animals used for developmental and reproduction tests.



***In vitro* genotoxicity**
Participants were "encouraged to use *in vitro* genetic toxicity testing… unless known chemical properties preclude[d] its use." These highly sensitive tests, OECD TG 471 and 473, for bacterial reverse mutations and *in vitro* mammalian chromosome aberrations are internationally accepted. Despite this clear guidance, 33 *in vivo* genotoxicity tests for chromosomal aberrations – requiring 2310 animals – were proposed in original test plans. EPA objected to only 12 of these tests and as a result, 26 *in vivo* genotoxicity tests – requiring 1820 animals – were proposed in latest test plan revisions.

Fish Acute Toxicity
Several thoughtful toxicology measures can reduce the number of fish used for ecotoxicity testing. In its guidance document, *The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*, EPA describes the use of SAR to estimate ecotoxicity values, noting that the established SAR-based computer program ECOSAR may be used to estimate toxicity to fish with an appropriate rationale for its applicability.[6] Also, in a December 2000 Federal Register Notice, EPA stated that for chemicals determined to have a log K_{ow} equal to or greater than 4.2, the fish acute toxicity test should generally be replaced with a chronic study in *Daphnia*, since this test is more likely to give a meaningful measure of ecotoxicity for these extremely hydrophobic chemicals. Finally, there is no reason to test insoluble, corrosive or reactive chemicals on fish.

We estimate that 2,850 fish were saved as a result of the use of these thoughtful toxicology measures in original test plans. PETA and PCRM called for these measures to be applied in at least 29 more cases – which would have saved another 1,740 fish. However, EPA often claimed that their use was unjustified, resulting in an additional 1,320 fish being required in latest test plan revisions.

References

[1]OECD Test Guidelines available at: http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html.
[2]Letters to Manufacturers/Importers available at: http://www.epa.gov/oppt/chemrtk/pubs/general/ceoltr2.htm.
[3]Data Collection and Development on High Production Volume (HPV) Chemicals available at: http://www.epa.gov/HPV/pubs/update/ts42213.htm.
[4]OECD SIDS Manual Sections 3.4 and 3.5 available at http://www.epa.gov/oppt/chemrtk/pubs/general/sidsappb.htm.
[5]Sandusky CB, Even M, Stoick K, Sandler J. Strategies to Reduce Animal Testing in US EPA's HPV Programme. *ALTEX*. 2006;23 Suppl:150-2.
[6]The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program available at: http://www.epa.gov/oppt/chemrtk/pubs/general/sarfinl1.htm.
[7]Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council. *Toxicity Testing in the 21st Century: A Vision and a Strategy.* 2007. THE NATIONAL ACADEMIES PRESS, Washington, D.C. 216 pp.
[8]Strategic Plan for Evaluating the Toxicity of Chemicals available at: http://www.epa.gov/osa/spc/toxicitytesting.