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U.S. High Production Volume Chemicals Challenge Program

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Abbreviations: ACC (American Chemistry Council); API (American Petroleum Institute); APO (animal protection organization); BPA (Benzene Phosphinic Acid); CAD (chromosomal aberration/damage); ChAMP (Chemical Assessment and Management Program); CSI (closed system intermediate); EDF (Environmental Defense Fund); EPA (U.S. Environmental Protection Agency); FDA (U.S. Food and Drug Administration); GRAS (Generally Recognized as Safe); HPV (high production volume); HPVIS (high production volume information system); K_{ow} (n-octanol/water partition coefficient); NIH (U.S. National Institutes of Health); NOAEL (No Observed Adverse Effect Level); OECD (Organisation for Economic Co-Operation and Development); PETA (People for the Ethical Treatment of Animals); PCRM (Physicians Committee for Responsible Medicine); PHTG (Petroleum HPV Testing Group); (Q)SAR (quantitative structure activity relationship); REACh (Registration, Evaluation, Authorization and Restriction of Chemical Substances); RSTP (Robust Summaries and Test Plans); SIDS (Screening Information Data Set); SNUN (significant new use notification); SNUR (significant new use rule); TG (test guideline); TSCA (Toxic Substances Control Act); WoE (weight of evidence).

ABSTRACT

Background: Launched by the U.S. Environmental Protection Agency (EPA) in 1998, the High Production Volume (HPV) Challenge Program (the Program) was developed to address the perceived gap in basic hazard information for the 2,800 chemicals produced or imported into the U.S. in quantities of one million pounds or more per year. Health and environmental effects data obtained from either existing information or through new vertebrate animal testing were voluntarily submitted by chemical companies (sponsors) to EPA. Despite the potential for extensive animal testing, animal welfare guidelines were not provided until after the start of the program.

Objectives: We evaluated compliance with the animal welfare principles that arose from an agreement reached between EPA and animal protection organizations (APOs) and tracked the Program's use of animals for testing.

Discussion: Under a worst case scenario, the Program had the potential to consume 3.5 million animals in new testing. After application of animal saving measures, approximately 127,000 were actually used. Categorization of chemicals based on similar structure-activity and application of read-across, along with use of existing test data, were the most effective means of reducing animal testing. However, animal saving measures were inconsistently employed by both sponsors and EPA.

Conclusions: Lessons learned from the HPV Program can be applied to future programs to minimize animal testing and promote more human-relevant chemical risk assessment.

INTRODUCTION

Background

Launched in October 1998 as part of the Chemical Right-to-Know initiative (U.S. EPA 1998b), the High Production Volume (HPV) Chemicals Challenge Program (the Program) was developed by the U.S. Environmental Protection Agency (EPA) in concert with the non-governmental environmental advocacy group, Environmental Defense Fund (EDF), the American Petroleum Institute (API), and the Chemical Manufacturer's Association [now American Chemistry Council (ACC)]. The Program focused on chemicals produced in or imported into the U.S. in annual quantities of one million pounds or more, which in 1998 amounted to approximately 2,800 substances. The stated goals of the Program were to collect health and environmental effects data and provide the public with basic hazard information on these chemicals that would allow individuals to actively participate in environmental decision-making (U.S. EPA 2011e).

Chemical companies were encouraged to volunteer for the Program or face regulation under the Toxic Substances Control Act (TSCA) (15 U.S.C. §§ 2601 et seq. 1976). For each chemical that was sponsored, EPA requested all the information specified in the Organisation for Economic Cooperation and Development (OECD) HPV Screening Information Data Set (SIDS) (OECD 2012). The OECD, as one of its functions, sets international standards and publishes validated methodologies for chemical safety testing. Developed for the OECD HPV Chemicals Programme, SIDS consists of physico-chemical information and data on environmental fate/pathways, ecotoxicity, and mammalian toxicity (U.S. EPA 2010a).

Animal Testing and Introduction of Animal Saving Measures

To satisfy SIDS ecotoxicity and human health effects data requirements that relied on animal data (endpoints), a chemical sponsor could either submit existing animal test results or conduct new animal tests. Considering the 2,800 chemicals identified and the amount of test data sought, the Program had the potential to consume millions of animals in new testing efforts, yet there had been no participation by animal protection organizations (APOs) in its planning. Subsequent critiques of the Program by People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), other non-governmental organizations including the Doris Day Animal League, the American Anti-Vivisection Society, and the Medical Research Modernization Committee, and the public, eventually led to an agreement with the White House and EPA to include a number of animal protection measures in the Program (e.g. Hess 1999; Lazaroff 1999; PETA 1999). This agreement set a precedent in the government's incorporation of animal welfare concerns into federal testing requirements.

The agreement was issued in the form of a letter (U.S. EPA 1999), sent by EPA in October 1999 to all participating companies, which outlined the new guidelines for animal use. Program participants were directed to: (1) not perform an animal test when a validated non-animal method was reasonably and practically available; (2) use existing, scientifically adequate data to the maximum extent, including information from international chemical databases; (3) employ *in vitro* genetic toxicity testing unless known chemical properties precluded its use; (4) conduct a thoughtful, qualitative analysis, including consideration of a substance's physico-chemical

properties; (5) apply a “weight-of-evidence” (WoE) approach whenever possible and forgo conducting certain tests if appropriate; and, (6) maximize grouping of related chemicals into categories based on structure-activity relationships. In addition, sponsors were told to not develop sub-chronic or reproductive toxicity data for closed system intermediates (CSIs), which are defined as chemicals used to produce another chemical that are handled in ways that result in a low possibility of exposure, and to consider whether any additional information obtained through new testing would be useful or relevant for substances Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA). Finally, EPA agreed to incorporate these elements into future HPV test rules (U.S. EPA 1999).

Thus, in theory, several means existed at the start of the Program to satisfy the health and environmental effects endpoints requiring animal data while also meeting the goal of minimal animal use in testing. These animal saving measures are summarized in Table 1.

The HPV test battery included a total of six vertebrate animal-based endpoints, five for human health effects (acute toxicity to mammals, repeated dose toxicity, reproductive toxicity, developmental toxicity, and genetic toxicity) and one for environmental effects (acute toxicity to fish) (U.S. EPA 2000). Table 2 summarizes the vertebrate animal tests available to satisfy these endpoints, their OECD test guideline (TG) identification numbers, and the number of animals associated with each test. In general, EPA recommended combined protocols (TG 421 or TG 422) be used to screen multiple endpoints (U.S. EPA 2000); these tests require approximately half the number of animals called for in the separate developmental toxicity (TG 414) or reproductive toxicity (TG 415) tests. For the genetic toxicity endpoint, which includes gene

mutation and chromosomal aberration/damage (CAD), EPA recommended the use of *in vitro* assays for gene mutation. For CAD, sponsors could use either the *in vitro* TG 473 or the *in vivo* TG 474, but were asked to provide a rationale for proposing the animal test instead of the *in vitro* assay. One chemical tested for all animal test endpoints would require, on average, 60 fish and up to 2,480 mammals (Table 2) if separate repeated dose, developmental and reproductive toxicity tests, and the *in vivo* CAD test, were used.

Objectives

As of January 2010, 428 test plans had been submitted by sponsors to EPA to address 1,420 of the original 2,800 HPV chemicals, most of which were grouped in categories containing two or more related chemicals. The plans present existing data that satisfy some or all SIDS requirements, and propose new tests to fill any perceived data gaps. Here we review these plans and accompanying documents, with a primary focus on compliance with the principles contained in the animal welfare guidance, and the resulting impact of proposed and actual tests on animal use. We also determine which animal saving measures were most effective for reducing the number of animals used.

Methods

We reviewed all publicly available test plans, EPA comments on those test plans, and test plan revisions accessible through the Robust Summaries and Test Plans (RSTP) table on EPA's HPV Challenge Program website (U.S. EPA 2012d). While EPA commented on 413 of the 428 test

plans submitted, sponsors subsequently revised only 330. We based our analysis on original test plans, EPA comments, and test plan revisions available on the website as of January 2010.

For each health and environmental effects endpoint potentially requiring animal test data, we determined whether new animal testing was proposed in original test plans and if not, which of the animal saving measures listed in Table 1 was used to satisfy the endpoint. If new animal testing was proposed, the OECD TG to be followed was noted, or if none was specified, we assumed that the tests listed in program guidance (U.S. EPA 2000) were to be used, i.e., TG 203 for acute toxicity to fish, TG 425 or TG 403 for acute mammalian toxicity, TG 421 for developmental and/or reproductive toxicity, TG 422 if repeated dose along with developmental and/or reproductive toxicity were proposed, and TG 473 for CAD (Table 2). We tallied the final number of each type of animal test or animal saving measure used by recording how each endpoint was addressed in the most recent document posted on the EPA website (U.S. EPA 2012d), i.e., either: (1) the original test plan, if no EPA comments on that test plan or subsequent revisions were posted; (2) EPA comments on the original test plan if no subsequent revisions were posted; or (3) the latest revision posted in response to EPA comments.

After reviewing original test plans, EPA generally indicated one of the following for each animal test proposed by sponsors: (1) that the test was accepted as proposed; (2) that the proposed test was unnecessary; (3) that the proposed test could be replaced with a different test requiring fewer or no animals. The agency also recommended additional tests in cases where it did not accept one or more animal saving measures proposed by sponsors to satisfy required endpoints. When sponsors responded by submitting revised test plans, we noted whether or not they agreed to

make the changes recommended by EPA. If sponsors had not responded, we counted the tests recommended in the EPA comments.

In some cases involving complex mixtures and process streams, chemical companies proposed new testing for related, non-HPV substances rather than for the sponsored chemicals themselves. We included the tests used for these substances in our animal test totals, and endpoints for the sponsored chemicals were counted as satisfied by read-across, a process by which endpoint information for one chemical is used to predict the same endpoint for another chemical based upon similarities in their chemical structure or functionality. In several cases for which sponsorship of chemicals was withdrawn due to overlap with international regulatory programs, we considered the endpoints addressed by existing data. In some situations EPA accepted or rejected proposed animal savings measures based on the sponsor meeting certain conditions, such as supplying study details in a robust summary or locating additional studies. If sponsors did not make revisions to test plans or it was unclear as to what was actually done, we had to judge whether those conditions were likely to be met and how the endpoints were eventually satisfied.

DISCUSSION

Analysis of Animal Use

Based on the median or standard number of animals used per test (Table 2), approximately 3.5 million animals would have been required to conduct a complete OECD SIDS battery on the 1,420 chemicals sponsored in the Program, using separate tests for each endpoint. This estimate

is reduced to about 994,000 animals if combined protocols were used for repeated dose/reproductive/developmental toxicity endpoints, instead of separate tests. Due to the use of animal saving measures the actual number of animals killed was substantially reduced, but still amounted to nearly 127,000, as explained in detail below.

In Figure 1, we summarize the extent to which the animal saving measures listed in Table 1 and new animal tests were used to satisfy all of the health and environmental effects endpoints potentially requiring animal test data for the 1,420 sponsored chemicals. Placing chemicals into categories and applying read-across from animal tests already conducted or proposed for analogous chemicals satisfied 55% of these endpoints. Submittal of existing test data also reduced animal use considerably, satisfying 27% of the endpoints. Such extensive availability of data for analogous chemicals and existing test results contrasts sharply with findings in the two reports that were largely responsible for the creation of the Program. In the 1997 publication *Toxic Ignorance* (EDF 1997), EDF stated "...today, even the most basic toxicity testing results cannot be found in the public record for nearly 75% of the top volume chemicals in commercial use." Likewise, EPA reported a paucity of data in its follow-up publication on chemical hazard data availability, *What Do We Really Know About the Safety of High Production Volume Chemicals?* (U.S. EPA 1998a). In another study (PCRM 1998), however, PCRM found that EDF and EPA had overlooked many databases containing toxicological data drawn from a wide variety of sources; these databases were later listed in EPA's October 1999 letter (U.S. EPA 1999) as available for use by sponsors.

Practical considerations – mainly cases in which sponsors determined a chemical to be ineligible for testing based on its physico-chemical properties – saved animals as well, avoiding testing for 9.2% of the endpoints (Figure 1). This approach was exemplified by butyllithium, a chemical described by its sponsor, FMC Corporation, as extremely reactive with air, moisture and animal tissues (FMC 2002). FMC further concluded that exposure of butyllithium to test animals would be cruel and would not generate meaningful data since the test animals would most likely have to be killed for humane reasons long before the end of the study (FMC 2002). In its comments on the test plan, EPA agreed with the unsuitability of butyllithium for SIDS testing, stating that “...owing to the highly reactive nature of this chemical when in contact with air or water, it is not feasible to perform physicochemical, environmental fate, mammalian or ecotoxicological tests” (U.S. EPA 2002a).

Non-animal methods, such as the *in vitro* CAD test, were used sparingly, accounting for only 1.2% of endpoints. A chemical’s status as a CSI satisfied requirements for <1% of endpoints (Figure 1).

Data requirements for the remaining 6.6% of endpoints were met by conducting new animal tests. Of the 334 tests proposed by sponsors in original test plans, EPA accepted 223, rejected 49 as not needed, and substituted 62 tests that used fewer or no animals (Table 3). Most of the substitutions involved replacing *in vivo* CAD tests with *in vitro* assays, and using combined protocols (TG’s 421 or 422) to evaluate reproductive and/or developmental toxicity, with or without repeated dose toxicity. EPA’s recommendations regarding elimination of tests and

replacement with less animal-intensive tests would have resulted in nearly 36,000 fewer animals used than originally proposed (Table 3).

Due to the voluntary nature of the program, and stated commitments to certain product stewardship efforts, however, sponsors did not always comply with EPA's recommendations to eliminate tests or to substitute tests using fewer/no animals. Sponsors went on to conduct 12 of the 49 tests EPA determined were not needed, including five fish toxicity tests using 300 fish, four acute mammalian toxicity tests using about 60 rodents, and three combined protocol tests using 1,740 animals. Sponsors also did not perform 21 of the 62 less animal-intensive tests recommended by EPA and, instead, conducted the tests originally proposed, i.e., five repeated dose tests (200 animals), eight developmental tests using TG 414 (4,640 more animals), three reproductive tests using TG 415 (1,740 more animals), and five *in vivo* CAD tests (250 animals). Thus, nearly 9,000 more animals were used in the Program as a result of sponsors' failure to follow EPA's recommendations. Of these, 6,380 could have been spared had combined protocols been used instead of separate reproductive or developmental toxicity tests.

During its review of test plans, EPA also recommended 154 additional tests be conducted, which, based on the median or standard number of animals per test in Table 2, would have used 57,000 animals. Of these 154 tests, sponsors agreed to conduct 75 (44 combined protocols, 29 acute fish, one developmental and one *in vivo* CAD) using about 28,500 animals, and declined to conduct 79 (46 combined protocols, 29 acute fish, three acute mammal, and one repeated dose), saving about 28,500 animals. For reasons not necessarily related to the Program, sponsors also added 43 tests (13 acute fish, nine combined protocols, eight repeated dose, seven *in vivo* CAD,

three acute mammal, two reproductive, and one developmental) after EPA review that used approximately 10,200 animals.

We estimated the net effect of EPA review and sponsor response on animal numbers by comparing the number of animals required for the original 334 test plans submitted by sponsors to the number required according to the most recent documents available on the EPA website (U.S. EPA 2012d) (Table 4). While the total number of tests increased from 334 to 349 after EPA review, the number of animals used decreased by about 3,000 to 126,460. This decrease was due not only to EPA's recommendations to eliminate tests or to substitute tests with fewer/no animals, but also to sponsors' declining to conduct additional testing recommended by EPA and, in a few cases, to sponsors' decisions to drop tests they had originally proposed. If *all* of EPA's recommendations had been followed, there would have been a net increase of 21,000 animals used, because while the agency's rejection of tests as unnecessary and recommendations to conduct tests using fewer/no animals would have reduced animal numbers by about 36,000 as noted above, the 154 additional tests requested would have increased animal usage by about 57,000.

Compliance with the Animal Welfare Agreement

An early review of the Program by PCRM (Cardello 2001) documented serious flaws in test plans submitted by sponsors including: (1) failure to report existing hazard information and to group structurally or toxicologically similar chemicals; (2) proposed animal tests that were beyond the scope of the Program; and, (3) lack of enforcement by EPA of agreed-upon animal

welfare principles. A subsequent evaluation of the Program by Nicholson et al. (2004) showed that many of the same problems reported by Cardello (2001) still existed. In addition, they found that testing was proposed for chemicals with known toxicities and for irrelevant endpoints when the primary hazard was high and well known, and that testing *in vivo* was proposed when valid *in vitro* methods were available.

In our analysis, we note inconsistencies both in EPA's treatment of the information submitted by sponsors and in sponsors' adherence to the animal welfare guidelines. For example, similar to the case made by FMC for butyllithium, the sponsor for benzene phosphinic acid proposed no additional mammalian toxicology testing because existing animal data showed that administration by oral gavage causes gastrointestinal tract bleeding, necrosis, and occasionally perforation, and the corrosive effects of this substance had already been demonstrated as the basis for its toxicity (BPD/BPA Coalition 2003). In its review, EPA cited a 14-day repeated dose study conducted in 1981 (Haskell Laboratories 1982) using dietary exposure that found lower doses of benzene phosphinic acid did not appear to cause animals distress and recommended a combined repeated dose/reproductive/developmental toxicity test (TG 422) be done despite the animal welfare concerns of the sponsor (U.S. EPA 2004). The final Robust Summaries Report (a robust summary describes the objectives, methods, results, and conclusions of a full study in enough detail to allow a technically qualified person to make an independent assessment of that study) for benzene phosphinic acid (BPD/BPA Coalition 2004) indicated that the sponsor conducted a new oral repeated dose study (28-day using TG 407) in 2003, which showed essentially the same NOAEL result (779 mg/kg males; 859 mg/kg females) as the 1981 study

(863 mg/kg). To satisfy the reproductive/developmental toxicity endpoints, however, the sponsor used data from a 1996 test on a similar substance, toldimfos.

Even with recommendations by EPA to use existing information, some chemical sponsors still failed to summarize all available data and instead proposed animal tests. For its Fuel Oils Category, ACC (2001) proposed evaluation of acute aquatic toxicity with two fish tests (using 120 fish), despite already possessing data on this endpoint for similar products. In addition, ACC acknowledged in its test plan that these substances consist of neutral organic hydrocarbons, whose toxic mode of action is well understood to be non-polar narcosis. When these fish tests were conducted on two representative oils in 2004 (ExxonMobil Biomedical Sciences, unpublished data), the LC₅₀ and LL₅₀ results were within the range of acute fish toxicity data already reported by ACC for this category in its original test plan. Moreover, in the final Robust Summaries for this category (ACC 2005), ACC cited two 1998 fish studies (Targia and Freeman, unpublished data) performed with No. 2 fuel oil that were apparently overlooked in 2001 when the original test plan was prepared.

Contrary to its own guidance to "...conduct a thoughtful, qualitative analysis rather than use a rote checklist approach" (U.S. EPA 1999), EPA sometimes applied a more narrow definition of program requirements when it rejected existing toxicity and exposure data and instead recommended new animal tests. This was evident in EPA's call for an acute fish test for the Mononitrile Category (U.S. EPA 2003), despite the sponsor's determination that no additional testing was needed based on the combined evaluation of data from several existing fish and invertebrate studies (Brooke et al. 1984; Dupont Haskell Laboratory, unpublished data),

application of the predictive computer program ECOSAR (U.S. EPA 2011c), the physico-chemical characteristics of the compounds, and the limited potential for meaningful aquatic exposures (Dupont 2002, 2004). Another example is EPA's treatment of the Ionone Derivatives Category, substances which naturally occur in plants containing β -carotene. The Flavor and Fragrance Consortia, which sponsored this category, cited studies (Stofberg and Kirschman 1985; Stofberg and Grundschober 1987) that showed human exposure is more likely via consumption of fruits and vegetables than by consuming products flavored with these substances, and noted that Ionone Derivatives are recognized by FDA as GRAS for their intended use in food (Flavor and Fragrance Consortia 2002). Based on these factors and existing data from studies already conducted, no new animal tests were proposed, yet EPA recommended a new developmental toxicity test that uses more than 1,000 animals (U.S. EPA 2002b). Rather than conduct the new test, the sponsor provided in its revised test plan (Flavor and Fragrance Consortia 2004), a more comprehensive analysis of data from a 1986 developmental study on hamsters (Willhite 1986) that had already been cited in the original test plan.

Grouping related chemicals into categories offers a means for not only reducing the number of new animal tests required but also for providing a contextual basis from which to evaluate toxicity. Of the 428 original test plans reviewed by EPA, 125 are for categories of related chemicals accounting for 1,117 of the 1,420 sponsored chemicals. Yet, additional opportunities to group related chemicals into categories were missed, resulting in duplicative and inefficient testing strategies. For example, tris(nonylphenol) Phosphite, sponsored by the Phosphite Producers HPV Consortium, could have been assessed in the context of a larger group of phenyl-

phosphorus antioxidant stabilizers, and p-cumylphenol, sponsored by General Electric, could have been included in a larger substituted or alkylphenol category (Cardello 2001).

Use of non-animal methods to reduce animal testing was not fully exploited despite the October 1999 guidance letter (U.S. EPA 1999) clearly stating that validated non-animal methods should be used whenever possible. The 96 *in vitro* CAD (TG 473) tests proposed in the most recent test plans or EPA comments did spare the lives of 4,800 animals, but 29 *in vivo* CAD (TG 474) tests were also proposed, with only six sponsors submitting the required justification for using this assay. EPA rejected one of the *in vivo* tests entirely, and in 13 cases recommended use of the *in vitro* test instead (Table 3), yet 22 *in vivo* CAD tests still were performed, killing 1,100 animals (Table 4).

Another non-animal method with the potential to reduce animal use was the computer program ECOSAR (U.S. EPA 2011c), which predicts aquatic toxicity based on structure-activity relationships. While EPA described this (Q)SAR method as providing screening-level characterization of ecotoxicity endpoints, including acute toxicity to fish, it still generally required fish test data from an analog to be summarized whenever ECOSAR was used (U.S. EPA 2010d), severely limiting the potential of the model to reduce fish use. This limitation appears to contradict EPA's own use of ECOSAR estimates, as described on its website (U.S. EPA 2011c): "The U.S. EPA Office of Pollution Prevention and Toxics uses SARs to predict the aquatic toxicity of new industrial chemicals in the absence of test data. Environmental assessors, chemical manufacturers, chemical suppliers, and other regulatory agencies have used ECOSAR to develop quantitative screening level toxicity profiles." Sponsors substituted ECOSAR data in

place of animal tests only 29 times in the absence of analog test data, and EPA rejected 11 of the proposed substitutions.

EPA also recognized that chemicals with high n-octanol/water partition coefficients (K_{ow}) are less likely to be toxic to fish and recommended in its program guidance that a chronic toxicity to *Daphnia* study be conducted in place of acute toxicity to fish for chemicals with a $\log K_{ow} \geq 4.2$ (U.S. EPA 2000). Surprisingly, sponsors proposed 18 new fish tests for chemicals that met the K_{ow} criteria for use of *Daphnia* data and EPA accepted 16 of these test proposals, though sponsors subsequently dropped six of the proposed fish tests in test plan revisions.

While a substance's solubility in water should have been a primary consideration in determining whether to test for aquatic toxicity, fish testing was nevertheless conducted on substances with very low solubility. For example, in its test plan for rosin – a naturally occurring substance from pine trees used in chewing gum, printing ink, adhesives and coatings – and rosin salts, substances used in paper products, soaps and detergents (PCA 2001), the Pine Chemicals Association acknowledged that rosin was essentially insoluble in water. Yet, it went on to conduct acute toxicity tests on fish, *Daphnia* and algae, the results of which showed that none of the compounds in this category were toxic to aquatic organisms (PCA 2004).

Choosing one of the combined protocols, TG 421 or 422, which each use 580 animals (Table 2), to screen for reproductive and developmental toxicities had the potential to save many animals compared to conducting separate tests for these endpoints, which each use 1,160 animals. Sponsors initially proposed 129 combined protocols, potentially saving between 75,000 and

225,000 animals, depending on whether the combined test replaced one or both of the separate tests. EPA recommended TG 421 or 422 tests in place of 24 proposed TG 414 and six proposed 415 tests (Table 3), but only 17 of these recommendations were accepted. Nevertheless, combined tests had a significant impact on reducing the number of animals used in testing HPV chemicals.

Sponsors cited a substance's physical, chemical or biological properties as a reason for precluding animal testing for 561 endpoints. However, some sponsors still proposed animal tests even when a chemical's properties rendered the results of these tests meaningless. In its initial test plan for the Petroleum Gases Category, API's Petroleum HPV Testing Group (PHTG) proposed separate acute mammalian, repeated dose, reproductive, and developmental toxicity tests on each of the individual gases ethane, butane, propane, and isobutane, even though these gases are explosive at concentrations below those at which health effects are observed and have been shown to act primarily as simple asphyxiants (Nicholson 2004). After receiving comments from APOs and EPA, PHTG reconsidered its testing proposal and eliminated all acute mammalian tests and all separate reproductive and developmental toxicity tests on individual gases from its revised test plan (API 2001). However, it still went on to conduct combined protocol tests on the four individual gases, results of which showed no or very minor health effects (API 2009). Interestingly, PHTG's original plan called for no testing of methane due to its physico-chemical properties, and despite EPA's disagreement with this finding, PHTG refused to change its position (API 2001), maintaining that "...the physical properties and ubiquitous presence of methane in the environment (including being a metabolic product of intestinal bacteria in humans) make health effects testing on methane unnecessary."

Notwithstanding its own guidance that participants need not develop certain data for chemicals that were solely CSIs, EPA rejected 33 out of 74 sponsor claims that testing for repeated dose and reproductive toxicity was not needed based on a chemical's classification as a CSI. The agency often failed to give specific reasons for rejecting these claims, only listing the CSI requirements and stating that the information provided was inadequate to support them.

EPA agreed to consider a lack of effects on the reproductive organs observed in a 90-day repeated dose toxicity test as a means of satisfying the reproductive toxicity endpoint when a developmental toxicity study was also available, as provided for in OECD SIDs guidance (U.S. EPA 2010a). While the agency did reject two proposed reproductive toxicity (TG 415) tests on this basis, it went on to recommend new testing on 14 chemicals for which sponsors had submitted lack-of-effects data because, in most of these cases, the data submitted failed to fully meet EPA's established criteria (U.S. EPA 2010a) for waiving the reproductive toxicity test.

Despite EPA's stated willingness in its October 1999 letter (U. S. EPA 1999) to accept WoE as a reason for not testing – "...[p]articipants 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" – sponsors provided WoE arguments in only 76 cases and EPA rejected 34 of those claims.

Regulatory Efforts to Collect Data

In order to develop data on “orphan” chemicals – those that were not sponsored in the voluntary portion of the Program and for which EPA determined that data were still required – the agency began supplemental rulemaking under TSCA. Three TSCA Section 4 Test Rules were proposed, and later finalized (U.S. EPA 2006c; U.S. EPA 2011g; U.S. EPA 2011h), between December 2000 and October 2011. These rules required manufacturers to provide health and environmental effects data on 51 orphan chemicals that met Section 4 reporting criteria, i.e., the chemical is produced or enters the environment in substantial quantities or there is significant human exposure; existing data are inadequate for risk assessment; and testing is needed to develop the data required for the risk assessment (U.S. EPA 2011i). Comments supplied by APOs regarding these rules succeeded in eliminating or reducing animal testing for a number of chemicals (see Supplemental Material pp 2 – 3 for examples). In addition, EPA issued TSCA Section 8(a) and Section 8(d) data reporting rules (U.S. EPA 2006b; U.S. EPA 2006a) in August 2006 for 243 HPV chemicals, 35 of which were subsequently removed from the list of unsponsored substances subject to reporting under TSCA Section 8 (U.S. EPA 2006d; U.S. EPA 2007).

On October 21, 2011, EPA issued a proposal to collect data on 23 remaining HPV chemicals through a fourth and final TSCA Section 4 test rule (U.S. EPA 2011a). In the same notice, the agency also proposed to simultaneously issue a Significant New Use Rule (SNUR) under TSCA Section 5(a)(2) for another 22 HPV chemicals. The SNUR would require manufacturers to file Significant New Use Notifications (SNUN) with EPA prior to any uses of the listed chemicals that would result in significant consumer or occupational exposure. This exercise of its authority under TSCA Section 5 appears to allow EPA to effectively require new testing for HPV

chemicals, including animal testing, without first finding that there are insufficient data upon which to determine health or environmental effects, as required under TSCA Section 4. Such an approach would very likely lead to duplicative testing should the agency fail to comprehensively search for relevant data and provide opportunities for public review and comment. In addition, since companies producing the same chemical would likely cross the SNUN threshold at different times, and some may not cross it at all, the SNUR approach could lead to duplicative reporting requirements by defeating efforts to share costs and testing through formation of consortia.

Outcomes and Future of the HPV Challenge Program

Sponsors were asked by EPA to submit SIDS data no later than 2005 (U.S. EPA 2001), yet more than a decade since the start of the program new test plans and revisions are occasionally submitted, and EPA has posted information on its RSTP website (U.S. EPA 2012d) as recently as May 2012. A web interface for accessing the hazard data (U.S. EPA 2012e), the HPV Information System (HPVIS), was not launched until April 2006, and efforts to familiarize potential users of the data have been limited to one national data-users conference held in December 2006 (U.S. EPA 2010b), and two regional workshops held in 2007 (U.S. EPA 2010c). While several methods of data query are offered on the HPVIS website, there is considerable variability in format and presentation of the data (e.g., multiple or inconsistent units), which limits the ability to use this information. Clearly, data formatting requirements should have been standardized early in the program.

The utility of the HPV dataset for risk assessment is limited, acknowledged even by EDF, the organization that strongly advocated for the formation of the HPV Program, as “...provid[ing] little if any reliable, comprehensive information about the use of and exposure to HPV chemicals” (ED 2007). EPA has used some of the data in its now obsolete Chemical Assessment and Management Program (ChAMP) to develop screening-level hazard, exposure, and risk characterizations for certain HPV chemicals (U.S. EPA 2012a). As part of its effort to identify and appropriately regulate chemicals of concern, EPA has also produced action plans for ten chemicals or groups of chemicals (U.S. EPA 2012b), two of which, bisphenol A and the nonylphenol/nonylphenol ethoxylates group, are produced in high volumes, although identification of these substances as chemicals of concern does not appear to have been a direct result of data collection under the Program.

The voluntary nature of the Program and the limited data acquisition authority of EPA under TSCA have led to a lengthy and fragmented data-gathering process, and attempts to update TSCA to address this problem have, thus far, been unsuccessful, as discussed in Supplemental Material. While it is appropriate to tailor data acquisition to meet regulatory needs, information also should be obtained in an organized, efficient manner.

In the fourteen years since the Program began, numerous new methods, initiatives and programs have been launched that promise to set priorities, reduce animal testing, and provide better regulation in the long run. Driven in part by the realization that animal testing is inefficient and that the information it provides is often difficult to use for regulatory purposes, the National Academy of Sciences published a seminal report, *Toxicity Testing in the Twenty-first Century: A*

Vision and a Strategy (National Research Council 2007), which describes a novel and rational approach to chemical safety assessment and the reduction of whole animal testing. EPA embraced this approach in its Strategic Plan for Evaluating the Toxicity of Chemicals (U.S. EPA 2009) and in the realignment and consolidation of several of its programs into the Chemical Safety for Sustainability Research Program (U.S. EPA 2011b). Furthermore, EPA, the National Institutes of Health (NIH) and the FDA have invested heavily in a “Tox21” collaboration to develop the technology necessary for this new approach (e.g., NIEHS 2011). These strategies and tools are designed to provide more relevant information faster and less expensively than what can be achieved with the current animal-based approach. They are already being incorporated into some of EPA’s chemical safety programs as evidenced by the agency’s “Pesticide Program Vision for Enhancing Integrated Approaches to Testing and Assessment” (U.S. EPA 2011f), changes to its Existing Chemicals Program Strategy (U.S. EPA 2012c), and the recent announcement of EDSP21 (U.S. EPA 2011d; U.S. EPA 2012g), EPA’s new work plan for its Endocrine Disruptor Screening Program.

Considering the length of time it has been in existence and the increased number of chemicals that now meet the HPV definition, the Program as administered by EPA was clearly unable to keep pace with changes in the chemical industry. An industry-led initiative, Extended HPV (EHPV), was announced in 2005 (e.g., Sissell 2005) to expand the Program to include the 574 chemicals that had reached HPV levels since its start. Although some EHPV data have been submitted to EPA, with the inception of the European Union’s mandatory Registration, Evaluation, Authorization and Restriction of Chemical Substances (REACH) regulation (European Commission 2006) in 2007, many global manufacturers and importers of chemicals

have shifted their focus to developing data for REACh in the hope that the same data can be used to meet U.S. requirements. Notably, many of the chemicals included in the fourth TSCA Section 4 proposed test rule mentioned above are either already registered under REACh or preregistered for the May 31, 2013 deadline. In comments on this test rule (ACC 2012), the chemical industry expressed concern over duplication of reporting requirements and called for EPA to formally harmonize its test guidelines with those of OECD, accept robust summaries of data submitted under REACh, and finalize a data sharing agreement with the European Chemicals Agency (ECHA) begun with the signing of a Statement of Intent in December 2010 (ECHA 2010).

Compared to the threshold of one million pounds for the HPV Program, REACh is decidedly more ambitious as its goal is to comprehensively assess the safety of all chemicals produced or imported in Europe in quantities of one tonne (2,205 pounds) or more (European Commission 2006). REACh prioritizes chemicals for testing by manufacture or import volume, and data requirements increase as the manufacture or import volume increases. The enabling legislation (European Commission 2006) contains language emphasizing the minimization of animal use and includes some measures corresponding to those implemented in the HPV Program, such as grouping of chemicals and use of read-across. Moreover, a stated objective of the legislation is to promote non-animal test methods, and it provides a list of accepted alternative methods and other means of avoiding animal testing. However, a drawback to including specific testing methods is that it can make it more difficult to adopt new methods as they become available, as exemplified by the current debate (e.g., ECHA 2011b) over the legality of replacing the two-generation reproductive toxicity test (OECD TG 416) with the new extended one-generation test (OECD TG 443) that reduces the number of animals used by half.

REACH guidance also includes detailed descriptions of integrated strategies that can be used to minimize testing and increase efficiency (e.g., ECHA 2008). Like the HPV Program, actual efficiencies and reductions in animal use will depend on the degree to which these animal saving measures are implemented. A recent preliminary assessment (ECHA 2011a) of the use of animal alternatives in the first phase of REACH indicated that formation of consortia by chemical companies greatly reduced duplicative animal testing and, as we observed for the Program, the use of existing data and read-across satisfied the largest number of endpoints requiring vertebrate animal testing. Not surprisingly, considering the current scarcity of universally accepted non-animal tests, data from only three *in vitro* methods – eye irritation, skin irritation and genotoxicity – were submitted. This is likely to change as non-animal assessment tools, such as the (Q)SAR models collected in the OECD Toolbox (OECD 2010), continue to be developed and implemented.

CONCLUSIONS

The U.S. HPV Challenge Program had the potential to consume more than three million animals in health and environmental effects testing, but after involvement by APOs, a variety of animal saving measures were introduced that reduced the number of animals actually used to approximately 127,000 – still a considerable amount. Grouping related chemicals and applying read-across to estimate the toxicity of untested chemicals had the greatest impact on reducing animal use. Discovery by both APOs and chemical sponsors of existing data also substantially decreased testing on animals, a significant finding considering that the Program was founded on

the premise that little hazard assessment data existed for HPV chemicals. Non-animal methods such as *in vitro* tests and computer simulation had comparatively little impact on mitigating animal use, satisfying only about one percent of the endpoints potentially requiring animal test data.

Of the animal tests that were conducted, combined protocols that could assess multiple endpoints in a single test significantly reduced animal use. Sponsors proposed these much more often than separate tests, and EPA, in its test plan reviews, went on to recommend them in place of nearly all the separate tests proposed.

Because participation in the Program was voluntary and HPV sponsors may have had other reasons for conducting tests, EPA's recommendations to eliminate tests or conduct tests involving fewer/no animals were not always followed, thus resulting in almost 9,000 more animals being used. On the other hand, the voluntary nature of the Program saved animals by allowing sponsors to decline to do additional testing recommended by EPA, although this testing may still be required through regulatory means at some point in the future. Ultimately, the impact of the Program on animals could have been far greater if APOs and other members of the public had not succeeded in advancing basic animal welfare principles shortly after it began.

The Program's primary goal of making chemical hazard information available to the public seemingly has been met by the posting of raw data on EPA's website (U.S. EPA 2012f). However, EPA significantly underestimated the amount of time necessary to complete the program, and its own use of the data to assess the hazards of HPV chemicals has, for the most

part, not progressed beyond the screening level stage. While the data can be retrieved digitally, albeit through a somewhat cumbersome web interface, the extent to which the public is using it to participate in environmental decision-making is unknown. Also, the Program did not systematically address information requirements by standardizing data reporting.

Both the HPV and REACh programs showcase the need for applying a different approach to prioritization of chemicals for further evaluation and articulating targeted data requirements to increase the efficiency of chemical risk assessment. The science of toxicology is evolving rapidly, and while only some of the tools being developed as part of the Tox21 collaboration have been adequately evaluated for use in risk assessment, they can and are being used for prioritization and screening purposes. Building on the means by which animal testing was reduced in the HPV Program along with the development of new technologies, will likely increase the efficiency and efficacy of chemical hazard and risk assessment and continue to decrease the use of animals in chemical safety testing. However, decision-makers must ensure incorporation of animal welfare principles and evolving chemical assessment strategies into current and future regulatory efforts in order for this to occur.

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Table 1. Animal saving measures available to satisfy health and environmental effects endpoints and minimize animal testing in the HPV Challenge Program.

Group	Animal Saving Measures
Existing Data	Submitting existing test results for specific SIDS endpoint
Read-across ^a	Grouping chemicals based on structure-activity relationships (SARs) and using read-across from tested chemicals to evaluate analogous untested chemicals
Practical Considerations ^b	Obviating tests based on physico-chemical or biological properties, exposure route, or use; observed effects from previous non-SIDS tests; reproductive toxicity satisfied by lack of observed effects on reproductive organs in a repeat dose test of 90 days or longer plus negative findings from an existing developmental toxicity study; WoE; GRAS substances; and other relevant information
Non-animal Methods	<i>In vitro</i> methods for genetic toxicity and quantitative (Q)SAR computer programs, such as ECOSAR, which estimates toxicity to fish, invertebrates and algae
Closed System Intermediates	Appropriately classifying chemicals as CSIs, and thereby avoiding the need for repeat dose toxicity and reproductive toxicity tests, which were not required for CSIs

^a Read-across is a process by which endpoint information for one chemical is used to predict the same endpoint for another chemical based upon similarities in their chemical structure or functionality.

^b EPA's October 1999 (U.S. EPA 1999) letter urged sponsors to conduct a "thoughtful, qualitative analysis." We termed the animal saving measures covered under this umbrella as "Practical Considerations."

Table 2. Animal tests used in the HPV Chemicals Challenge Program animal tests, OECD test guideline (TG) number, and numbers of animals associated with each test. Tests recommended by EPA in HPV Program guidance are indicated below.

TEST	OECD TG	Animals used [No. or median (range)]
Acute Toxicity Fish ^a	203	60
Acute Toxicity Mammal		
Oral, Up-and-Down Method ^a	425	10 (6 – 15)
Acute Oral ^b	401	23 (20-25)
Acute Inhalation ^a	403	23 (20-25)
Oral, Toxic Class Method	423	9 (6-12)
Repeated Dose Toxicity		
28-day Oral ^a	407	40
28-day Inhalation	412	53 (40-65)
90-day Oral	408	80
90-day Inhalation	413	80
Developmental Toxicity		
Prenatal Developmental Toxicity	414	1,160
Reproductive Toxicity		
One-Generation Reproduction	415	1,160
Combined Protocols		
Reproductive/Developmental Toxicity Screening ^a	421	580
Repeated Dose/Reproductive/Developmental Toxicity Screening ^a	422	580
Genetic Toxicity ^c		
Mammalian Erythrocyte Micronucleus CAD	474	50

^a Indicates test recommended in EPA HPV Program guidance (U.S. EPA 2000).

^b This test was deleted from the manual of accepted OECD test guidelines in 2002; however, it was included in a few early proposals.

^c EPA program guidance for the genetic toxicity chromosomal aberration/damage (CAD) endpoint was the *in vitro* TG 473.

Table 3. EPA's recommendations regarding animal tests originally proposed by sponsors, i.e., test accepted, test not needed, or test substituted with one using fewer/no animals, and potential number of animals saved.

Test	Tests Proposed in Original Plans	Test Accepted	Test Not Needed	Animals Saved ^a	Tests Using Fewer/No Animals	Animals Saved ^b
Acute Tox. Fish	87	77	10	660	NA ^c	-
Acute Tox. Mammal	26	7	17	170	2	26
Repeated Dose Tox.	23	2	4	160	17	892
Developmental Tox.	29	1	4	4,640	24	15,080 ^d
Reproductive Tox.	11	3	2	2,320	6	4,640 ^d
Combined protocols	129	118 ^e	11	6,380	NA ^c	-
CAD ^f (<i>in vivo</i>)	29	15	1	50	13	650
Total	334	223	49	14,320	62	21,288

^a Number of animals saved by eliminating tests, based on standard or median number of animals per test as shown in Table 2.

^b Number of animals saved by using tests with fewer/no animals, based on standard or median number of animals per test as shown in Table 2.

^c NA: not applicable, i.e., there was no substitute test for combined protocols, which already used fewer animals, or for the acute toxicity to fish test.

^d EPA recommended 22 combined protocols in place of either reproductive or developmental toxicity and four combined protocols in place of both reproductive and developmental toxicity. The animal savings by endpoint was calculated as follows: Dev. Tox. = 20 x (1160-580) + 4 x ((2320 - 580)/2) = 15,080; Rep. Tox. = 2 x (1160-580) + 4 x ((2320 - 580)/2) = 4,640.

^e For two tests, EPA made no comment; these tests were assumed to have been accepted by EPA.

^f CAD = Chromosomal aberration/damage

Table 4. Initial number of tests proposed and animals required versus final number of tests in most recent program documents (revised test plan or EPA comments) and animals required.

Test	Initial Number of Tests Proposed	Number of Animals Required ^a	Final Number of Tests	Number of Animals Required ^a
Acute Tox. Fish	87	5,220	111	6,660
Acute Tox. Mammal	26	440	16	185
Repeated Dose Tox.	23	1,172	15	755
Developmental Tox.	29	33,640	11	12,200
Reproductive Tox.	11	12,760	8	9,280
Combined protocols	129	74,820	166	96,280
CAD ^b (<i>in vivo</i>)	29	1,450	22	1,100
Total	334	129,502	349	126,460

^a Number of animals based on standard or median number of animals per test as shown in Table 2.

^b CAD = Chromosomal aberration/damage.

Figure Legend

Figure 1. Percentages of endpoints requiring animal data satisfied by animal saving measures or new animal tests for the 1,420 sponsored HPV chemicals. Note that for some chemicals animal tests were performed on non-HPV chemicals or mixtures of chemicals and the results of these tests were used to generate read-across for the HPV chemical. In these cases both the animal test and read-across were counted toward fulfilling the endpoint.

