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of OECD Working Party on Existing Chemicals
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HPV Chemical Human Health Testing: Animal Welfare Issues and Approaches

EPA is mounting a very extensive program to obtain toxicological screening information on chemicals of High Production Volume (HPV), that is, substances produced in excess of 1M lb/year. Current information indicates that there are about 2800 chemicals with that designation. Various parties have noted that if each chemical in the program were to be tested for each of the human health effects tests, a large number of animals would be employed. In recognition of these concerns, the Agency has given thought to the issue and is developing a strategy to reduce animal use while still generating needed high quality health information.

Many different paths are being investigated to ensure the minimization of animal usage and optimization of procedures for those animals that go into test in the HPV testing program:

1. Decreasing chemicals going into test

a. Industry will determine whether adequate information on chemicals already exists for the various endpoints. We do not want to retest chemicals.

b. EPA has released a data adequacy document which provides guidance on making such determinations. EPA is also in the process of developing guidance on procedures for searching the literature on other sources of existing information.

c. Both the OECD's HPV Program and the HPV Challenge in the U.S. encourage industry to develop categories of chemicals which can be assessed as a group. These categories of related chemicals are expected to share chemical and biological attributes. Instead of gaining information on all members of a category, attempts will be made to identify testing strategies that will identify individual materials which are representative of the category. By testing the identified individual materials, we should be able to characterize the potential fate and effects of the whole category.

d. Structure-activity relationships (SAR) will help to identify potential toxicities and other effects of individual chemicals based on Quantitative Structure Activity Relationships (QSARs) or "read-across' (i.e., analogue) approaches.

2. Minimizing and optimizing animal use in tests

The HPV testing program includes acquisition of health effects data for chemicals on acute toxicity, reproductive toxicity, developmental toxicity, 28-day repeated dose toxicity and mutagenicity. Mutagenicity data requirements can be fulfilled with bacterial gene mutation, in vitro mammalian cytogenetics (for pre-existing information) and in vivo micronucleus (for pre-existing or newly generated information). Several opportunities are available to evaluate the role of animals in testing and ensure that their use is being appropriately addressed.

a. **Replacement of animal testing**. In some cases we need not obtain health hazard information in animals. Mutagenicity testing can be fulfilled by bacterial systems (e.g., Salmonella gene mutation) and, in some cases, by cytogenetics in cultured mammalian cells.

b. **Refinement of animal testing**. EPA supports the employment of federal and voluntary measures to ensure humane care and upkeep of laboratory animals. In addition, we plan to utilize principles developed in an upcoming document on humane endpoints from OECD. This report will lay out signs of pain and stress in animals that should be utilized in deciding when to terminate animals in test.

c. **Reduction of animal testing**. There are several opportunities to reduce the number of animals committed to test. **Table 1** illustrates potential animal savings for the case where some or all health effects tests are performed on a chemical.

(1) **Acute toxicity**. There are 4 acute oral toxicity tests approved by OECD. In the use of the traditional test (OECD 401), about 30 animals are employed to screen for toxicity following a single exposure. Three alternative methods either refine or reduce animal usage. Data from any of the acute methods may yield appropriate information for HPV testing. Among the three alternative methods, EPA has identified a preference for the up-and-down method (OECD 425) for the following reasons: it greatly reduces the number of animals in comparison to OECD 401 (the up-and-down method uses approximately 8 animals versus 30 in OECD 401); it gives a point estimate of the LD50; and it yields information that can be used to estimate the toxicity of chemical mixtures in accordance with the UN transport classification system.

(2) **Reproductive and developmental toxicity**. There are separate test guidelines for 1-generation reproduction toxicity (OECD 415) and for prenatal developmental toxicity (OECD 414; revision of this test is ongoing at OECD). If separate reproduction and developmental toxicity tests were conducted using current OECD 415 and 414 protocols, 320 animals would be used. To screen for

reproductive and developmental toxicity and to reduce animal usage in comparison to the separate test guidelines, EPA recommends use of a combined toxicity protocol (OECD 421) for the U.S. HPV testing program.

(3) **28-Day repeated dose toxicity**. Instead of conducting a stand-alone 28-day oral toxicity test (OECD 407), the endpoints covered by that guideline can be combined with the reproduction/developmental toxicity screen into OECD 422 with no increase in number of animals over that used in OECD 421.

(4) **Mammalian micronucleus**. The traditional in vivo micronucleus test is performed using 2 sexes and a concurrent positive and negative control. EPA is exploring the idea of using at least the males from OECD 422 for all but the positive control. Females may need to be dosed separately.

(5) **Overall animal savings**. By selecting specific tests, there could be a significant savings in animals committed to test in the HPV program. If the traditional acute, reproduction, developmental and 28-day repeated dose toxicity studies and the in vivo micronucleus test were separately employed, a total of 440 animals might be used. By using alternative and combined test protocols, the number of animals could be reduced to 118, a savings of 322 animals (>70%) per chemical. Actually, the savings would be greater because most tests employ dose sighting studies.

Table 1. Potential reductions in animal usage in the U.S. HPV testing program

Human Health Toxicity Test (OECD #)	Sample Size (approx.)	Dose Sighting Study	Animal Savings Compared to Traditional Test (in bold)
ACUTE TOXICITY			
401 Acute oral toxicity	30	yes	
420 Fixed dose	20	yes	
423 Acute toxic class	9	no	
425 Up-and-down	8	no	22
REPRODUCTION/DEVELOPMENTAL TOXICITY			
415 One-generation reproduction toxicity	160	yes	
414 Teratogenicity	160	yes	
421 Reproduction/developmental toxicity screen	80	yes	240
28-DAY REPEATED DOSE TOXICITY			
407 Repeated dose 28-day oral toxicity	40	yes	
422 Combined repeated dose toxicity and reproductive/developmental toxicity screen	80 *	yes	40
MUTAGENICITY			
474 Mammalian erythrocyte micronucleus	50 2 sexes	yes	
422 Combined developmental toxicity screen with micronucleus test for males; females may need separate dosing.	30 2 sexes	yes	20
TOTAL ANIMALS REQUIRED **Without use of reduction strategies** **With use of reduction strategies**	 440 118		
TOTAL SAVINGS OF ANIMALS WITH USE OF REDUCTION STRATEGIES			322 (> 70% reduction)

* same animals as would be used in OECD 421