Estimating the Extent of the Health Hazard Posed by High-Production Volume Chemicals

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We used structure–activity relationship modeling to estimate the number of toxic chemicals among the high-production volume (HPV) group. We selected 200 chemicals from among the HPV chemical list and predicted the potential of each for its ability to induce a variety of adverse effects including genotoxicity, carcinogenicity, developmental, and systemic toxicity. We found a significantly less than expected proportion of toxic chemicals among the HPV sample when compared to a reference set of 10,000 chemicals representative of the universe of chemicals. *Key words*: high production volume (HPV) chemicals, prevalence, structure–activity relationships, toxicity. *Environ Health Perspect* 109:953–956 (2001). [Online 5 September 2001] *http://ehpnet1.niehs.nih.gov/docs/2001/109p953-956cunningham/abstract.html*

The TestSmart program is a collaborative project between the Johns Hopkins Center for Alternatives to Animal Testing, the Environmental Defense Fund, Carnegie-Mellon University, and the University of Pittsburgh (*1*). The TestSmart program was conceived in response to the U.S. Environmental Protection Agency's (U.S. EPA's) Chemical Right-to-Know Initiative High Production Volume (HPV) Chemical Challenge Program with the goal of providing a humane, economical, and efficient method of collecting basic toxicologic data for HPV chemicals (*2–4*). For this purpose, HPV chemicals are defined as those produced or imported into the United States in quantities greater than 1 million pounds per year. The program asks chemical producers and importers to voluntarily provide basic toxicologic data on HPV chemicals (*5*). These chemicals were identified under the Toxic Substance Control Act 1990 Inventory Update Rule (*6*). Overall, the HPV Chemical Challenge Program list contains 2,800 chemicals (*7*). The Screening Information Data Set (SIDS) of the Organization for Economic Cooperation and Development was selected as the toxicologic criteria needed to meet the goals of the HPV Chemical Challenge Program (*8*). SIDS includes tests for genotoxicity, acute and chronic toxicity, reproductive toxicity, ecotoxicity, and environmental fate.

One of the challenges, as part of the TestSmart Program, was to assess the overall magnitude of the health hazards posed by HPV chemicals based on structure–activity relationship (SAR) modeling. The U.S. EPA will consider test result submission for the HPV Program based on SAR models that are scientifically justifiable (*9*). As part of this program we undertook an analysis of a random set of 200 HPV chemicals and predicted the probability of each to induce a variety of toxic effects including genotoxicity, carcinogenicity, developmental toxicity, and systemic toxicity. The majority of these chemicals are not part of the learning sets used to derive the SAR models, thereby eliminating the possibility of tautological artifacts. Although SAR projections may not have perfect predictivity, the current study seeks to assess the prevalence of toxicants among HPV chemicals. Such estimates based on SAR techniques can be derived for populations of molecules provided the SAR model has been validated and its predictivity is known (*10–12*).

Materials and Methods

HPV chemical selection. A sample of 200 chemicals was selected from among the HPV chemicals (*7*). The chemicals chosen were randomly selected and *a*) were pure and unique substances; *b*) were organic; *c*) were nonpolymeric; and *d*) did not contain metals.

Reference chemicals. A reference set of 10,000 chemicals representing the universe of chemicals was used as a control set. The composition of this set is consistent with estimates produced by the National Academy of Science (*13*). This set was derived through sampling chemical structure libraries and the National Institutes of Health Developmental Therapeutics Program. This reference set was used to assess whether the HPV chemicals represent a greater or lesser toxicologic risk than other chemicals. For this evaluation we compared the percentage of chemicals predicted to be toxic in the HPV sample to the percentage of chemicals predicted to be toxic in the reference chemical set.

SAR predictions. We used the CASE/MULTICASE program (MULTICASE Inc., Beachwood, OH) (*14–16*) to predict the toxicity of the sampled HPV chemicals and the 10,000 chemicals in the reference set. All chemicals from both groups were predicted for their ability to induce a number of different toxic end points (Table 1). Each toxic end point was predicted separately. The predictions are based on the occurrence of identified molecular features that have been previously identified as significantly related to toxicity for each end point.

The CASE modeling process begins with the compilation of a set of chemical structures (typically in Smiles code) and an experimentally derived biological activity value. These data are placed into a learning set for the program. Each chemical in the learning set is broken down, *in silico*, to all possible fragments from 2 to 10 heavy (i.e., nonhydrogen) atoms. Each fragment is labeled with the name and activity of its parent chemical. Upon completion of this process, the program organizes the list of fragments and tabulates the number of chemicals containing each of them. The program then identifies those fragments that were identified predominantly in active chemicals and refers to these fragments as biophores. The selection of biophores is based on the binary experimental results of each chemical. For example, biophores for a cancer causation model are identified that are predominantly found in chemicals that tested positive for carcinogenicity compared to those that were noncarcinogenic. The particular potency value associated with each biophore is then determined from the experimental potencies for the chemicals making up the biophore. The total list of biophores is then used to derive a global quantitative SAR (QSAR) equation. These biophores serve as the basis for both predictive and mechanistic analysis of toxicity.

The MULTICASE module then selects from the list of biophores the most important one based on its occurrence in the largest number of chemicals in the learning set. At this point in the MULTICASE routine, a congeneric series of chemicals has been identified, with the biophore being the unifying feature. MULTICASE then performs a series of defined chemical substitutions of the atoms in the first biophore (e.g., one halogen for another halogen or a nitrogen for a carbon in aromatic systems) and then searches for these expanded definitions of the biophore in the

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library of previously identified significant fragments. All chemicals containing the biophore and the expanded definitions are grouped together. Thus a biophore may consist of a single feature or a family of chemically similar features.

Using the molecules contained in this family of chemicals as a new learning set, MULTICASE identifies modulators of their activity. These modulators may be chemical, physicochemical, or quantum mechanical parameters. Modulators augment or decrease the activity of the chemicals containing the biophore. Some values and coefficients are localized to particular atoms of a chemical (e.g., a charge or highest occupied molecular orbit coefficient on an individual atom derived by a modified Hückel method). The biophore and identified modulators are then used to derive local QSAR equations for chemicals within this subset. If the entire learning set is congeneric, then the single biophore and associated modulators may explain the activity of the entire set; this usually does not occur and there will be a group of molecules not explained by the single biophore and associated modulators. When this happens, the program will remove from consideration the molecules already explained and will search for the next biophore. The process is iterated until all of the active molecules in the learning set have been explained or until no significant fragments can be found to explain them.

The resulting list of biophores can then be used in mechanistic studies or to predict the activity of yet untested molecules (*10*). For example, upon submission for evaluation, MULTICASE will determine if an unknown molecule contains a biophore. If the molecule does not contain a biophore, it will be predicted, by default, to be inactive. When the molecule contains a biophore, the program will make a qualitative prediction that the chemical is biologically active with an associated probability that this prediction is correct. Moreover, MULTICASE will inspect the molecule for the presence of modulators associated with this biophore. The program then incorporates the parameters for the identified modulators into the QSAR equation and produces a quantitative prediction for the potency of the chemical. In essence, although biophores are the determining structures, the modulators will determine whether and to what extent the biological potential of the chemical containing the biophore is expressed.

Application of the CASE and MULTICASE programs results in four submodels (*17,18*). These are two models to estimate potency and two to estimate probability of activity. Because each of them may reflect different facets of the toxicologic phenomena under study, they are combined to give an overall Bayesian probability of the toxicity for each chemical tested. A chemical is considered active if its Bayesian probability is > 0.6 and negative if it is < 0.4.

SAR models. A number of validated and characterized SAR models (*11*) of toxicologic phenomena were used in the course of these studies. These included the induction of mutations in *Salmonella*. That database was developed under the aegis of the U.S. National Toxicology Program (NTP) (*19–23*). SAR models based on subsets of that database have been described (*24–26*). SAR models of the ability to induce error-prone DNA repair in *Escherichia coli* (SOS Chromotest; EBPI, Brampton, Ontario, Canada) (*27,28*), mutations in cultured mouse lymphoma cells (*29*), sister chromatid exchanges (SCEs), chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells (*30*), and unscheduled DNA synthesis in primary rat hepatocytes (*31*) have been described previously, as have models of the potentials for inducing SCEs (*32*) and micronuclei *in vivo* (*33*).

We used two rodent carcinogenicity databases: the Carcinogenic Potency Database (CPDB) assembled by Gold and associates (*34–38*); and the rodent carcinogenicity database generated under the auspices of the NTP (*39,40*). SAR models of these databases have also been described (*41–43*). We combined the individual projections derived from these different databases using Bayes' theorem, described previously (*17,18*) to yield a single prediction of carcinogenicity.

The SAR model of cellular toxicity was based on assays using cultured BALB/c-3T3 cells (*44*). A chemical was considered cytotoxic if its IC_{50} (concentration that inhibits 50% growth) value was ≤ 1 μM. The SAR model of lethality to minnows was derived from previously published data (*45*). The SAR model for lethality to rats (LD_{50}; 50% lethal dose) was based on data on 1,411 orally administered chemicals extracted from the Registry of Toxic Effects of Chemical Substances (*46*). In that SAR model, toxicity was defined as $LD_{50} \leq 7.2$ mmol/kg.

The SAR models for eye irritation (*47*), sensory irritation (*48*), developmental toxicity in humans (*49*) and in hamsters (*50*), allergic contact dermatitis (*51*), inhibition of gap junctional intercellular communication (GJIC) (*52*), and environmental biodegradation (*53*) were also used.

The SAR model of α_2u-globulin nephropathy in male rats (*54*) was based on data kindly supplied by L.D. Lehman-McKeenan from the Procter and Gamble Company (Cincinnati, OH).

The predictive ability of each model was estimated by its ability to correctly predict the activity of chemicals not used to build the model but for which we knew the true experimental results. These values are listed in Table 1 as concordance (i.e., percent correct predictions over total predictions). These values were calculated based on pooling multiple 10-fold cross-validation results. Each learning set was divided 10 times into learning and validation sets. Each learning set was used to derive a model, and this model was then used to predict the activity of the chemicals left out in the validation set. Because the activity of the chemicals in the validation set was known, we could determine the number

Table 1. Predicted prevalences of toxicants among groups of chemicals.

SAR model	Prevalence (%) HPV chemical	Universe of chemicals	*p*-Value	Concordance (%)
Mutagenicity: *Salmonella*	19.5	31.5	0.0001	85
SOS DNA repair (Chromotest)	3.0	12.4	< 0.0001	87
Mutagenicity: mouse lymphoma	19.0	49.2	< 0.00001	70
Unscheduled DNA synthesis	6.0	21.8	< 0.00001	78
Sister chromatid exchanges *in vitro*	29.0	17.1	< 0.00001	71
Chromosomal aberrations *in vitro*	19.5	33.4	< 0.00001	66
Induction of micronuclei *in vivo*	46.5	59.5	0.00001	81
Sister chromatid exchanges *in vivo*	45.5	56.8	0.0007	83
Carcinogenicity in rodents	16.5	33.5	< 0.00001	74
In vivo genotoxicants	8.0	23.0	< 0.00001	NA
Genotoxic carcinogens	4.5	16.0	< 0.00001	NA
Cell toxicity	26.5	41.2	< 0.00001	84
α_2u-Globulin mediated nephropathy	8.5	14.4	0.009	83
Inhibition GJIC	14.0	27.3	< 0.00001	70
Developmental toxicity: hamsters	18.0	26.3	0.004	74
Developmental toxicity: humans	3.0	16.4	< 0.00001	75
Allergic contact dermatitis	31.0	48.4	< 0.00001	86
Sensory irritation	19.5	43.4	< 0.00001	79
Eye irritation	33.0	47.4	0.00003	80
Lethality to rats	15.0	84.7	< 0.00001	84
Toxicity to minnows	38.0	56.4	< 0.00001	76
Biodegradability	47.0	48.1	0.4	70

Abbreviations: GJIC, gap junctional intercellular communication; NA, not applicable because these are joint probabilities based on two SAR models (*11*).

of correct predictions and estimate the concordance for each model.

Results and Discussion

The HPV chemicals can be considered to present an elevated toxicologic risk to humans and to the environment based solely on their large production volume and the consequent potential for exposure (*55*). However, it would be of interest to know whether the HPV chemicals, as a group, are more or less toxic than "average" chemicals. To assess this, we compared the proportion of chemicals in the HPV sample predicted to be toxic to the proportion of chemicals predicted to be toxic in the reference set representing the universe of chemicals. These comparisons were done one toxic end point at a time. Unexpectedly, for all toxic effects assessed except one (the *in vitro* induction of SCEs), the proportion of chemicals predicted to be toxic among the HPV sample was significantly less than the proportion of chemicals predicted to be toxic in the reference set (Table 1). The question obviously arises as to the reason for this decrease in the number of potentially toxic HPV chemicals when compared to what would be expected from a random sample of chemicals. This is particularly relevant given that the underlying reason for the HPV Challenge program is that little is known about the toxicities of the HPV chemicals (*55*). From this reasoning, it can be assumed that hazardous chemicals were not excluded from production based on the results of toxicologic prescreens.

A more detailed analysis of the mutagenic/genotoxic potentials indicate that with respect to the possibility for inducing mutations in *Salmonella*, the proportion of HPV chemicals predicted to be mutagens was significantly less than that for the reference set (19.5% vs. 31.5%, $p = 0.0001$; Table 1). Interestingly, it has recently been reported that of 46 HPV chemicals tested for *Salmonella* mutagenicity, 20% were mutagens (*56*). Moreover, this same report showed an increase in the proportion of mutagens when comparing HPV chemicals to all chemicals in commerce. This is in concordance with our predictions.

Predictions based on other assays designed to assess mutagenic and genotoxic activity in prokaryotes or cultured cells (Table 1) showed the same pattern; (i.e., the proportion of HPV chemicals predicted to induce these effects was lower that for the chemicals in the reference set). The only exception to this is the proportion of chemicals predicted to induce SCEs in cultured CHO cells. However, the ability to induce SCEs *in vitro* is not restricted to genotoxicants and may, in fact, reflect cell toxicity (*57*).

The *Salmonella* mutagenicity assay is usually the first screen used, but the results are frequently confirmed by an *in vivo* test for genotoxicity. The assay frequently used to confirm that *in vitro* assay is the mouse micronucleus assay (*58*). The proportion of chemicals predicted as *in vivo* micronucleus inducers among the HPV sample is also significantly less than that for the reference set (Table 1). The same is true for the other *in vivo* assay, the induction of SCEs in mice (Table 1). It should be noted that although the micronucleus assay is confirmatory when the *Salmonella* assay indicates the potential for mutagenicity, the micronucleus assay response can also be elicited by nongenotoxicants such as inhibitors of tubulin polymerization and of microtubular integrity, as well as by aneugens (*59,60*). This may explain the greater projected proportion of micronuclei inducers when compared to *Salmonella* mutagens.

Based on predicted positive responses in both the *Salmonella* mutagenicity and the micronucleus assays, which define *in vivo* genotoxicants, we estimate that 8% of the chemicals in the HPV sample possess that potential, in contrast to 23% of chemicals in the reference set (Table 1), thus further suggesting that the HPV chemicals, as a group, represent less of a genotoxic risk than chemicals at large.

The major function of many mutagenicity and genotoxicity assays is to help identify carcinogens that may pose a risk to humans (*58*). Based on predictions made by several SAR models derived from rodent carcinogenicity data, the HPV sample is estimated to be significantly less likely to induce cancers than the reference chemicals (16.5% vs. 33.5%, $p < 0.00001$; Table 1). However, these proportions are based on rodent cancer bioassays in which animals are exposed up to the maximum tolerated dose for their lifetime. It is doubtful that this is an apt model for human exposure. On the other hand, the majority of recognized human carcinogens are also mutagenic and/or genotoxic (*61–63*). To evaluate the prevalence of genotoxic carcinogens (*39*), we predicted the proportion of chemicals that would induce cancers in rodents and mutagenicity in *Salmonella* (i.e., genotoxic carcinogens). Again, there was a significant decrease in the proportion of chemicals predicted for these end points between the HPV sample and the reference set (4.5% vs. 16%, $p < 0.00001$; Table 1).

Carcinogenicity in rodents is not solely due to DNA damage. Other mechanisms including cell toxicity, mitogenesis, and inhibition of GJIC have been postulated (*64–71*). For these end points also, the proportion of chemicals predicted positive in the HPV sample was less than that of the reference set (e.g., cell toxicity, 26.5% vs. 41.2%; α_2u-globulin–mediated nephropathy leading to renal tumors in male rats, 8.5% vs. 14.4%; and inhibition of GJIC, 14% vs. 27.3%; Table 1).

The HPV sample is predicted to have a lower proportion of chemicals that are developmental toxicants for hamsters or humans (Table 1). That sample was also predicted to have a lower proportion of inducers of allergic contact dermatitis, sensory irritation, and eye irritation (Table 1). Finally, the HPV sample was predicted to have a much lower proportion of systemic toxicants than the reference set (Table 1).

With respect to environmental effects, the HPV sample was predicted to contain significantly fewer aquatic toxicants than the reference set (Table 1). However, the estimated environmental biodegradability of the two groups was not significantly different.

Conclusion

In this study we predicted the occurrence of chemicals capable of inducing 10 separate toxicologic end points in a sample of HPV chemicals and compared these values to those from a reference set of 10,000 chemicals. Regardless of the nature of the toxicologic phenomenon, the subset of HPV chemicals was estimated to contain a significantly lower proportion of toxicants than the reference set.

Although it can be expected that the potential for human contact with the HPV chemicals is great, the potential for individual members of the group to induce health effects is less than expected. The reason for this lower proportion of toxicants in the HPV sample and presumably in the entire HPV list is unknown. However, it may reflect chemical properties of this group that allow them to be used as chemical stocks. These would include greater stability and lower reactivity, two useful properties for storage and transport of chemicals. Thus the HPV chemicals, for utility and handling ease, are not typically reactive. Presumably these chemicals, during the processing to final products, are transformed into multiple reactive intermediates in less than HPV quantities.

During the course of this study, Zeiger and Margolin (*56*) estimated the proportion of mutagens in a subset of HPV chemicals using preexisting data. Their results matched ours, leading us to conclude that our sample of HPV chemicals is representative of the group and that our predictions are in accord with experimental results.

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