Remarkably, the DNA sequence of any two people is 99.9 percent identical. The differences are expressed as variations in traits such as blood type and eye color. Differences in DNA sequences can also affect the degree to which an individual is at risk of developing an illness or other adverse response to environmental stressors.

Pharmaceutical researchers have long recognized the importance of genetic differences among individuals when they evaluate a drug’s effectiveness and side effects. With full sequencing of the human genome completed in 2003 and the revolution in new analytical and automated technologies, genomic information is becoming available at rates never seen before. Environmental toxicologists are using this information to help them identify genetic variations—known as polymorphisms—that play an important role in determining why people react differently to chemicals. As researchers learn more about genetic factors that contribute to differences in chemical sensitivity, their findings may be used to improve the accuracy of chemical risk assessments that are applied in the development of regulatory decisions for the protection of public health. In the U.S. Environmental Protection Agency’s (EPA) dose-response assessments for non-cancer health effects from exposure to chemicals, variability among humans is traditionally addressed by applying a default uncertainty factor. The estimated safe exposure level for an average human is divided by a number, usually 10, to ensure the level will be protective of sensitive individuals. If there are significant unknowns about the sensitivity of children, the uncertainty factor could be even higher.

An LRI-sponsored project led by Dr. Michael Dourson of Toxicology Excellence for Risk Assessment (TERA) and Mr. Harvey Clewell of ENVIRON International Corporation relied on a team of scientists from both companies to research approaches to evaluating differences in metabolism due to genetics. The goals were to develop a method that enables risk assessor to use genetic information for populations reliably in their chemical risk assessments and to identify instances where the traditional uncertainty factor approach can be replaced by methods that are based on scientific data.

**Differences in Metabolism Affect Risk**

Some individuals metabolize chemicals more slowly than others due to their genetic makeup. As a result, these individuals may be more or less sensitive to a chemical, depending on factors such as how the chemical or its metabolites (breakdown products) affect the body. For example, if a metabolite is less toxic than the original chemical, individuals with a faster metabolism will be more protected.

This project evaluated the effect of genetic differences in metabolism on the predicted blood concentration of warfarin (Gentry et al. 2002). Individuals carrying the slow metabolizing form of the gene are predicted to have more warfarin in their blood compared with individuals who carry the more prevalent forms of the gene (see graphic). Using the results of the method demonstrated with this research, a risk assessor can calculate how internal doses of a particular chemical are likely to be distributed across the population. Thus, an assessor can better quantify sensitive members of the population.

### Three forms of CYP2C9 gene that affect warfarin metabolism enzyme

- **Form 1**
  - codes for amino acid arginine at position 144 of enzyme protein
  - 78% of population
  - 157 mg/L plasma
- **Form 2**
  - codes for amino acid cysteine at position 144 of enzyme protein
  - 13% of population
  - 273 mg/L plasma
- **Form 3**
  - codes for amino acid leucine instead of isoleucine at position 359 of enzyme protein
  - 9% of population
  - 2,670 mg/L plasma

* assuming ingestion of 1mg/kg body weight of warfarin
The research team focused on the process of chemical metabolism, in which the body changes the original chemical into another form, called a metabolite. The default uncertainty factor for human variability includes a subfactor that addresses differences in either metabolizing (activating) the chemical to a more toxic form, or metabolizing (deactivating) it to a safer form. However, this default factor is imprecise, and can be replaced by chemical-specific information on metabolism. The purpose of the project was to develop a more accurate approach that incorporates information on how genetic differences affect metabolism. To do this, the researchers first conducted literature surveys to identify genes that are known to code for metabolizing enzymes that exist in more than one genetic form in the general population. The researchers then identified substances that are known to be metabolized by these enzymes.

They further evaluated four chemicals for which the metabolic pathways were well characterized (e.g., the key metabolizing enzymes were known), where the effect of genetic differences on metabolism were understood, and for which physiologically based pharmacokinetic (PBPK) models (i.e., models of how a chemical is absorbed, metabolized, distributed, and excreted) were available. They conducted detailed case studies with two of these chemicals, parathion and warfarin. Parathion is an organophosphate pesticide that was used widely until 1991, when its use was significantly restricted. Production of parathion was cancelled voluntarily in 2002 based on human and ecological risk concerns. Warfarin is used therapeutically as a blood “thinner” (an anticoagulant with a common name of coumadin) and has also been used as a rodenticide. Using PBPK models, the researchers estimated the concentrations of parathion, warfarin, and their metabolites in blood following a single oral dose of the compounds. The team used statistical techniques (probabilistic Monte Carlo methods) to evaluate the extent to which both common genetic differences and physiological differences (e.g., body weight, blood flow rate) contributed to the overall variation in internal dose for the population as a whole. The models provided estimates of the distributions of levels of the active form of the chemical, supporting efforts to predict how many people would exhibit higher, lower, or average sensitivity to the chemicals due to genetic differences.

The researchers found that the effects of genetic polymorphisms can vary widely from chemical to chemical. The modeling suggested that genetic differences in the metabolism of parathion make only a minor contribution to the overall variation in internal dose across a population, whereas genetic differences in warfarin metabolism account for a significant portion of the total variability. The researchers then compared the estimated extent of metabolic variation with EPA's default uncertainty factors, using methods developed by the International Programme on Chemical Safety (IPCS) to incorporate information on a chemical’s toxicokinetics in the development of estimates of “safe” doses.

The results of this research are very significant for chemical risk assessment, because many chemicals regulated by various regulatory agencies are metabolized through pathways that are influenced by genetic differences. Data on the incidence of these genetic differences in human populations and their effects on metabolic pathways are becoming more readily available. This research demonstrates that data on polymorphisms can be combined with other information to improve the accuracy of the human variability uncertainty factor applied by EPA and other regulatory agencies in their chemical risk assessments for non-cancer health effects. The research identifies the data needs for supporting such analyses, and confirms the usefulness of probabilistic (Monte Carlo) applications of PBPK modeling in determining variations in the sensitivity of populations based on differences in metabolism.

EPA's policy is that its exposure limits should be protective of almost all of the population. Risk assessors frequently use EPA’s default uncertainty factors to represent differences in metabolism, regardless of whether information is available on metabolic variability. The work performed by Dourson et al. identifies opportunities both for revising the default values and for using chemical-specific data on genetic differences to more accurately represent variations in metabolism. Application of these techniques can help EPA and industry target their efforts to reduce exposure to the chemicals that are expected to have the greatest impact on the health of the general population.

Evaluations of human variability in response to specific toxic chemical exposures remain complex and controversial. The project described here identifies data needs and demonstrates a successful, systematic method for analyzing how human genetic differences may affect susceptibility to a particular chemical. The findings of the study will also support the development of a decision framework for incorporating information on genetic differences, which will help in prioritizing resources for data collection and analysis. The research also will improve cancer risk assessments, because the approach enables risk assessors to use data on genetic differences to more accurately predict tissue doses for populations.

Finally, this work points the way to a future when risk assessments will routinely identify segments of the population likely to be affected at different exposure levels. The studies summarized here will greatly facilitate the use of data on genetic differences in such probabilistic analyses.


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