THE SIRC REVIEW

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New Light on Some Key Health Issues

In this second issue of *The SIRC Review* we continue our examination of questions periodically raised—by government, industry, health specialists or other groups—with respect to the possible effects of exposure to styrene. Few chemicals have been so extensively studied and the scientific literature is vast. To help guide us through it, we have invited independent researchers—recognized authorities in their respective fields—to review and analyze the spectrum of relevant studies and offer their conclusions. The publication of these interpretive reviews in an accessible format for researchers and decisionmakers in industry and government is a major purpose of *The SIRC Review.*

The current issue provides us with comprehensive reviews of the literature in two notably important areas of inquiry: the potential for chromosomal aberrations and the possible effects of exposure on human reproduction. Both areas have been mentioned with various degrees of concern by some researchers, health and environmental groups.

Dr. Julian Preston of the Oak Ridge National Laboratory, who reviewed the literature on mutagenicity in our last issue, here reviews the assays on cytogenetic effects for evidence of chromosomal damage. He finds that much of the existing confusion in this area has arisen from the poor quality of so many of the studies, whose conclusions have nevertheless been taken at face value. The better quality studies generally conclude that styrene does not induce chromosome changes. While this is encouraging, Preston believes the question is important enough that more definitive research should be undertaken to fully clarify the issue.

Dr. Nigel Brown of London University examines the numerous investigations of reproductive toxicity, including the originals of several Soviet studies, but finds no clear evidence that styrene exposure has had adverse effects. Indeed, he found that some of the studies which did report reproductive effects were later contradicted—by the same researchers. Upon further research, they simply found that their earlier conclusions had been wrong.

Both reviews represent significant additions to our knowledge, and we are grateful to their authors.

Two other distinguished scientists in this issue are Dr. Bruce Ames and Dr. Lois Swirsky Gold of the University of California at Berkeley, who have been doing pioneering work in exploring the body's resistance mechanisms to what they describe as the "enormous background" of natural carcinogens in our environment—including, they remind us, at least 16 different varieties in our morning cup of coffee.

They conclude that we have gradually developed effective defenses against low levels of exposure to both natural and synthetic toxics. This is particularly pertinent in view of the growing interest in low exposure outcomes among researchers in some areas of the federal government and some private foundations, who express concern that we simply do not know what dangers we may be facing from low level exposures. Ames and Gold respond that what we don't know probably isn't worth worrying about because the "danger" may well be zero. A reassuring counterpoint.

They also argue that much of our current regulatory dependence on the results of rodent experiments may well be rendered irrelevant by new advances in understanding the mechanisms of carcinogenesis.

By far the most dramatic article in this *Review* is the scholarly and exceptionally precise critique of the federal government's risk assessment process by no less an authority than the Office of Management and Budget. Many of us who have had occasion to query the risk assessments issuing from government from time to time have assumed, deep down, that the regulators probably knew what they were talking about. Not so, says OMB in this quite devastating piece. Risks are often exaggerated by many orders of magnitude. Risk assessments are rife with policy judgments masquerading as science. The conservative (worst-case) bias routinely used for risk assessments gravely distorts the results and thus, too, the government's priorities. The result, says OMB, is that this flawed process may actually be increasing health and safety risks rather than reducing them.

It is the first time, to our knowledge, that anyone has pulled together so many examples of distortions—many

of them apparently quite deliberate—in what should be a totally objective, scientific risk assessment process. Several of the examples cited only an insider could know. When we launched the *Review,* we did not expect to be dealing in exposés. But this thoroughly researched document, which we borrowed with permission from a rather forbidding tome entitled *The Regulatory Program of the United States Government,* surely falls into that category—and is certainly very disturbing to those of us working in the field. The authors remind us (and those in government to whom their remarks appear to be addressed) of the sound guiding principles on risk assessment and risk management laid down in 1983 by the National Academy of Sciences, and also of the prescient warning of former EPA Administrator William D. Ruckelshaus that "nothing will erode public confidence faster than the suspicion that policy considerations have been allowed to influence the assessment of risk." We hope you will find this issue of *The SIRC Review* interesting as well as informative. In our next issue we plan to publish an important interpretive review of the principal neurotoxicity studies by Nicola Cherry of McGill University and a summary of the epidemiological investigation of some 16,000 workers in the reinforced plastics and composites industry by Otto Wong of ENSR Health Services.

Geoffrey C. Granville
Chairman
Science and Technology Task Group

Authors in this Issue

Dr. Bruce N. Ames, professor of biochemistry and molecular biology at the University of California, Berkeley, has long been recognized as a leading researcher in the field of carcinogenesis and gene regulation. He is internationally renowned as developer of the "Ames test" for the detection of mutagens and potential carcinogens. His most recent work has been in identifying the important mutagens damaging human DNA and the natural defense mechanisms of the body. He is a member of the National Academy of Sciences and was a member of the National Cancer Advisory Board from 1976 to 1982. He has been the recipient of numerous awards for cancer research and was awarded the prestigious Tyler Prize for Environmental Achievement in 1985. After serving as section chief in the Laboratory of Molecular Biology at the National Institutes of Health, he joined the faculty of the University of California at Berkeley in 1968. He currently directs the university's Environmental Health Sciences Center. He earned his Ph.D. in biochemistry and genetics at the California Institute of Technology.

Dr. Nigel A. Brown is internationally recognized for his work in reproductive and developmental toxicology and teratology. He is currently head of teratology at the Medical Research Council Experimental Embryology and Teratology Unit of St. George's Hospital Medical School at the University of London, where he also serves on the faculty. Previously he was with the George Washington University Medical School in Washington, DC, the National Institute of Environmental and Health Sciences at Research Triangle Park in North Carolina, and the University of Mainz in the Federal Republic of Germany. He was a founder of the Reproductive Toxicology Center in Washington, DC, and is a committee member of the US Teratology Society. He also serves as European Editor of the professional publication, *Reproductive Toxicology.* He is a council member of the European Toxicology Society and the British Developmental Pathology Society and has served on the scientific committee of the British Toxicology Society. He earned his Ph.D. in biochemistry at the University of Surrey in England.

Dr. Lois Swirsky Gold, director of the Carcinogenic Potency Database Project at the University of California's Lawrence Berkeley Laboratory, has collaborated with Dr. Ames (q.v.) in several investigations of carcinogenesis, including comparisons of naturally-occurring and synthetic chemicals, ranking possible hazards to humans, and the role of mitogenesis in carcinogenesis. She is currently working on a comparison of target organs in rats and mice and an update of worker exposures to rodent carcinogens. Dr. Gold is in the Environmental Health Sciences Center at the University of California, Berkeley, and is a member of the peer review panel that evaluates the cancer tests of the National Toxicology Program. She earned her Ph.D. at Stanford University and subsequently taught research methods at the University of California.

Dr. R. Julian Preston, Section Head of the Biology Division of Oak Ridge National Laboratory in Oak Ridge, Tennessee, is well known for his work in genetics, cytogenetics and molecular biology. In addition to his academic and research work, he is a member of the Toxicology Study Section of the National Institutes of Health, and a consultant in the areas of genetics and molecular biology to the Radiation Effects Research Foundation. He has also been associated with the US Environmental Protection Agency as a group leader for a gene-tox group studying cytogenetics. He has served on the editorial boards of *Mutation Research Letters, Mutation Research, Environmental and Experimental Botany* and *Environmental and Molecular Mutagenesis.*

Dr. Preston is an honors graduate of Britain's Cambridge University (genetics) and holds a Ph.D. in radiation biology from Reading University. Since 1970 he has been an adjunct professor at the University of Tennessee's Biomedical Graduate School and was associate director of the school from 1977 to 1982.

Risk Assessment and Risk Management: Current Regulatory Issues and Concerns

An Overview and Analysis

From the Regulatory Program of the United States Government, published by the Office of Management and Budget

This excerpt from the recently published Regulatory Program of the United States Government, prepared by the Office of Management and Budget, is a major review of problem areas in the federal government's current risk assessment and management practices. It points out that by continued reliance on worst-case assumptions and by incorporating hidden policy judgments within the scientific assessments of risk, the government has departed significantly from the recommendations of the National Academy of Sciences (NAS) and seriously distorted the risk process. The subsequent distortions are often of several orders of magnitude and are probably most severe in the area of cancer-risk assessment.

Many Federal agency regulatory decisions are intended to reduce risks to human life and health. Government regulations control which agricultural chemicals may be used to reduce insect damage, increase farm yields, and improve the quality of food products. Other rules govern hazards in the Nation's workplaces and emissions from its factories. There are regulations directing the way in which automobiles must be manufactured, commercial aircraft maintained, and trains operated. Hardly any widespread human activity that entails risk is free of some degree of social control, often achieved through government regulation.

Regulatory decisions involving risk require agencies to address questions such as, "How safe is 'safe'?" and "How clean is 'clean'?" When government agencies promulgate regulations intended to reduce a risk or mitigate a hazard, they are engaging in what has become known as *risk management*. These policy choices inevitably involve consideration of both the risks entailed by the underlying activity and the social consequences of regulatory intervention. Thus, the first challenge of risk management is to set priorities to determine which risks are worth reducing and which are not.

For government to carry out its risk-management responsibilities, there must be an extensive investment in the careful assessment and quantification of risks. The term *risk assessment* means the application of credible scientific principles and statistical methods to develop estimates of the likely effects of natural phenomena and human activities.

The need to keep risk assessment and risk management separate has long been the objective of responsible public officials. In 1983, the National Academy of Sciences (NAS) studied the process of managing risk in the Federal Government and offered the following recommendations, among others:

Recommendation 1: Regulatory agencies should take steps to establish and maintain a clear conceptual distinction between assessment of risks and the consideration of risk management alternatives, that is, the scientific findings and policy judgments embodied in risk assessments should be explicitly distinguished from the political, economic, and technical considerations that influence the design and choice of regulatory strategies.[21]

Recommendation 2: Before an agency decides whether a substance should or should not be regulated as a health hazard, a detailed and comprehensive written risk assessment should be prepared and made publicly available. This written assessment should clearly distinguish between the scientific basis and the policy basis for the agency's conclusions [22].

The belief that risk assessment and risk management should be kept separate enjoys widespread support among professional risk-assessment practitioners and risk-management officials.[23] Others have emphasized the importance of ensuring that policy biases do not distort

the analysis of alternative risk-management choices.[24] The NAS principles have also have been endorsed by a number of Federal agencies, including the Office of Science and Technology Policy (OSTP), the Environmental Protection Agency (EPA), and the Department of Health and Human Services (HHS).[25]

Unfortunately, risk-assessment practices continue to rely on conservative models and assumptions that effectively intermingle important policy judgments within the scientific assessment of risk. Policymakers must make decisions based on risk assessments in which scientific findings cannot be readily differentiated from embedded policy judgments. This policy environment makes it difficult to discern serious hazards from trivial ones, and distorts the ordering of the Government's regulatory priorities. In some cases, the distortion of priorities may actually increase health and safety risks.

This section explores some of the continuing difficulties that plague the practice of risk assessment, and describes briefly their policy implications. It can be summarized in three observations:

The continued reliance on conservative (worst-case) assumptions distorts risk assessment, yielding estimates that may overstate likely risks by several orders of magnitude. Many risk assessments are based on animal bioassays utilizing sensitive rodent species dosed at extremely high levels. Conservative statistical models are used to predict low-dose human health risks, based on the assumption that human biological response mimics that observed in laboratory animals. Worst-case assumptions concerning actual human exposure are commonly used instead of empirical data, further exaggerating predicted risk levels.

Conservative biases embedded in risk assessment impart a substantial "margin of safety". The choice of an appropriate margin of safety should remain the province of responsible risk-management officials, and should not be preempted through biased risk assessments. Estimates of risk often fail to acknowledge the presence of considerable uncertainty, nor do they present the extent to which conservative assumptions overstate likely risks. Analyses of risk-management alternatives routinely ignore these uncertainties and treat the resulting upper-bound estimates as reliable guides to the likely consequences of regulatory action. Decisionmakers and the general public often incorrectly infer a level of scientific precision and accuracy in the risk-assessment process that does not exist.

Conservatism in risk assessment distorts the regulatory priorities of the Federal Government, directing societal resources to reduce what are often trivial carcinogenic risks while failing to address more substantial threats to life and health. Distortions are probably most severe in the area of cancer-risk assessment, because many conservative models and assumptions were developed specifically for estimating upper bounds for these risks. Risk-assessment methods with similar conservative biases are less common elsewhere, particularly in those areas where real-world data are available, or where the mechanism by which injury or illness occurs is better understood.

A renewed commitment to the NAS recommendations is clearly warranted. As quantitative risk assessment plays an increasingly significant role in risk management, the need to separate science from policy becomes ever more important, if either process is to maintain public confidence. As former EPA Administrator William D. Ruckelshaus has noted:

> Risk assessment...must be based on scientific evidence and scientific consensus only. Nothing will erode public confidence faster than the suspicion that policy considerations have been allowed to influence the assessment of risk.[26]

ALTERNATIVE RISK-ASSESSMENT METHODOLOGIES

Risk assessments of chemical substances in general (and of possible carcinogens in particular) involve a mixture of facts, models, and assumptions. There is considerable debate concerning the scientific merits of the models and assumptions commonly used in risk assessments. In some cases, a scientific consensus has developed to support a particular model assumption. In other instances, however, certain models and assumptions are relied upon because they reflect past practices rather than the leading edge of science. Furthermore, a scientific basis for several of the most critical models and assumptions simply does not exist.

Most scientists agree that these models and assumptions impart a conservative bias: that is, they lead to risk projections that the actual (but unknown) risk is very unlikely to exceed. These "upper-bound" estimates are often useful as a screening device, to exclude from regulatory concern potential hazards that are insignificant even under worst-case conditions. Unfortunately, upper-bound risk estimates are routinely employed for altogether different purposes, such as estimating the likely benefits of regulatory actions. Policymakers are required to act on the basis of biased representations of both the magnitude of the underlying hazard and the extent to which Government action will ameliorate it.

Contemporary risk assessment relies heavily upon animal bioassay and epidemiology. Each approach has theoretical advantages and disadvantages. In practice, both

can be misused to bolster preestablished conclusions. The following discussion emphasizes problems in carcinogenic risk assessment, because the prevention and cure of cancer plays such a major role in policy issues involving risks to life and health.

Animal Bioassay

Animal testing enables scientists to estimate risks ex ante, before human health effects materialize, whereas epidemiological studies can only detect such effects ex post. In addition, animal tests can be conducted under tightly controlled laboratory conditions, which provide more reliable estimates of exposure and avoid many of the confounding factors that often plague epidemiological investigations. The relatively short lifetimes of experimental mammals (such as rats and mice) allow scientists to ascertain the possible effects of long-term exposure in just a few years.

Animal testing suffers serious limitations, however, arising from certain critical assumptions. Despite its routine application, there is no accepted scientific basis for the assumption that results can be meaningfully extrapolated from test animals to humans.[27] Some scientists believe that animal data should not be used in assessing human health risks.[28]

Another critical limitation is the reliance on very high doses to generate adverse effects in test animals.[29] A mathematical model must be used to bridge the gap between these high-dose exposures and the low-dose exposures more typically faced by people. Many different mathematical models can be constructed to fit the data at high doses. These models often vary enormously, however, in their predictions of risk at low doses.

Beyond these unavoidable methodological constraints, the results of animal bioassays may be subject to conflicting scientific interpretation or strongly influenced by the choice of research method. Tissue preparation and histology present obvious opportunities for error, as experts may disagree as to how slides should be interpreted.[3o] This problem generally is not significant at high doses, where malignancies are often obvious. At low doses, however, pathologists often differ in how they distinguish tumors from hyperplasia. Subjectivity cannot be avoided where such interpretations of the data must be made.[31]

Epidemiology

Epidemiology is attractive because it largely avoids these two problems. It focuses on observable human health effects instead of on hypothesized outcomes based on animal experimentation, and it relies upon real-world exposures to generate empirical data. Many of the serious problems associated with animal studies can be avoided, allowing researchers to develop risk estimates that are directly related to human health.

Unfortunately, epidemiological research suffers from its own set of limitations. For example, retrospective studies often have difficulty correlating morbidity and mortality with exposure to specific substances. Exposure data are commonly lacking, incomplete, imprecise, or affected by systematic recall or selection biases. Furthermore, the risks these studies seek to detect are often very small relative to background, thus making statistically significant effects difficult to observe. When health effects are latent, correlating exposures to illness is even harder.

Besides these unavoidable methodological limitations, epidemiological studies often suffer from outright bias. Many studies employ scientifically questionable procedures aimed at demonstrating positive relationships between specific substances and human illness.[32] Some researchers use inappropriate statistical procedures to "mine" existing databases in search of associations. One result of these practices is that epidemiological studies often display contradictory results?[33]

Despite these constraints, properly conducted animal bioassays and epidemiological studies both have useful roles to play in quantitative risk assessment. Indeed, they are complementary. The usual weaknesses of epidemiological investigations—unreliable exposure data, confounding effects—are readily avoided in laboratory experiments on animals. The weaknesses of animal bioassays—high to low-dose extrapolation, animal-to-man conversion—do not arise in epidemiological studies. Careful risk assessment incorporates both types of analysis to ensure that the emerging picture of human health risk is as complete as possible, and that inferences derived from this picture are themselves internally consistent.

ISSUES IN RISK ASSESSMENTS DERIVED LARGELY FROM ANIMAL BIOASSAYS

Animal bioassays tend to dominate current risk assessments. An important reason for this is that the derivation of dose-response relationships is a critical regulatory motive for performing quantitative risk assessment. Animal studies are ideally suited to serve this purpose by virtue of the controlled conditions under which dose and response can be calibrated. Epidemiological studies often are relegated to providing merely a "reality check" to ensure that the implications of animal bioassays are plausibly consistent with real-world experience. Because of this heavy emphasis on animal testing, the focus here is on several major problems that arise with respect to risk assessments

primarily based on the results of animal bioassays.

The Use of Sensitive Test Animals

To enhance the power of animal tests, scientists typically rely on genetically sensitive test animals. It is unclear whether these species accurately mimic biological responses in humans.

Some test species are extremely sensitive. For example, approximately one-third of all male $B_6C_3F_1$ mice, a common test species, spontaneously develop liver tumors.[34] The same phenomenon occurred in an important bioassay concerning dioxin using female Sprague-Dawley (Spartan) rats. Tumors observed in dosed animals were predominantly located in the liver. However, approximately one-fifth of the animals in the control group also developed liver tumors.[35] The relevance of elevated liver tumors in hypersensitive species has been questioned by scientists and is not universally considered probative evidence of carcinogenicity. Nevertheless, cancer risk assessments often proceed on the assumption that these data are sufficient to conclude that a substance is indeed a carcinogen.[36]

The reliance on sensitive test animals also biases risk assessments in a more subtle way. It establishes powerful incentives to search for and develop increasingly sensitive test species. As test animals become more sensitive, repeated testing using identical protocols will tend to result in higher and higher estimates of risk even if all other factors are held constant.

Selective Use of Alternative Studies

In their respective risk-assessment guidelines, both OSTP and EPA recommend that relevant animal studies should be considered irrespective of whether they indicate a positive relationship.[37] In practice, however, studies that demonstrate a statistically significant positive relationship routinely receive more weight than studies that indicate no relationship at all.[38] For example, the plant growth regulator daminozide (Alar) and its metabolite unsymmetrical 1,1-dimethylhydrazine (UDMIH) recently received B2 classifications ("probable human carcinogen"). Each of these classifications was based on a single positive animal bioassay.[39] Overcoming such a classification requires, at a minimum, two "essentially identical" studies showing no such relationship.[40] In the case of Alar and UDMH, however, a more stringent test was apparently applied: three high-quality negative studies showed no significant effects; these studies appear to have received little or no weight in the classification decision.[41]

Selective Interpretation of Results

Risk-assessment guidelines generally give the greatest weight to the most sensitive test animals. Thus, if a substance has been found to cause cancer in one species or gender but shown to exhibit no effects elsewhere, the results pertaining to the sensitive species or gender typically will be used to develop estimates of human-health risks. For example, if male mice develop cancer from a substance but female mice and rats of both genders do not, then the results from the male mouse often will be used to derive estimates of cancer risks to humans.[42]

Once a positive result has been obtained in an animal bioassay, a substance often will be provisionally classified as a probable human carcinogen. The statistical burden of proof then shifts to the no-effect hypothesis. Because it is logically impossible to prove a negative, however, this practice establishes a virtually irrebuttable presumption in favor of the carcinogenesis hypothesis.

Severe Testing Conditions

Current risk-assessment protocols require the use of very high doses. Unfortunately high doses are often toxic for reasons unrelated to their capacity to cause cancer. A common procedure is to use what is called the maximum tolerated dose (MTD), which is the most that can be administered to a test animal without causing acute toxicity. At such exposure levels, substances often cause severe inflammation and chronic cell killing. For example, formaldehyde causes nasal tumors in rats when administered in high doses. However, MTD administration severely inflames nasal passage tissues. It is therefore unclear whether the cancers induced are caused by formaldehyde per se or by the toxic effects of high doses.

Results such as these have caused some scientists to question the validity of rodent tests performed at the MTD for estimating human health risks that arise from exposure at low doses.[43] By combining very high doses with highly sensitive test subjects, some animal bioassays are predisposed to discover apparent carcinogenic effects.

Relevance of Animal Bioassay Results

An important reason why animals vary in their sensitivity is that they have different physiologies, metabolic processes, reproductive cycles, and a host of other species-specific characteristics that largely result from unique evolutionary paths. Each of these factors needs to be carefully considered in evaluating the significance of animal data with respect to human health. This is recognized in both the OSTP and EPA guidelines, but it is often neglected when the guidelines are applied to specific substances.[44]

The most important assumption in this regard is that animal test results can be meaningfully extrapolated to humans. A recent study of chemicals tested under the auspices of the U.S. National Toxicology Program shows that this assumption can lead to the erroneous classification of many chemicals as probable human carcinogens.[45] Positive associations have been obtained in either rats or mice for half of 214 chemicals tested. However, results were consistent across these two genetically similar species only 70 percent of the time. If it is assumed that rodent bioassays have the same sensitivity and selectivity with respect to human carcinogens as they do between rodent species, and it is further assumed that 10 percent of all chemicals are in fact human carcinogens, then 27 of every 100 randomly selected chemicals would be misclassified as probable human carcinogens. Only three chemicals would be misclassified as noncarcinogens. Thus, "false positives" would be 9 times more common than "false negatives."[46]

Of course, this ratio of false positives to false negatives reflects highly conservative "upper-bound" assumptions concerning sensitivity and selectivity. Given the high degree of similarity between rats and mice and the limited resemblance between rodents and humans, the sensitivity of rodent bioassays with respect to human carcinogenicity is probably much lower than 70 percent. Furthermore, other research indicates that selectivity may be as low as 5 percent. Adjusting only for this lower selectivity suggests that false positives are almost 30 times more common than false negatives. This raises serious questions concerning the practical utility of the current approach to animal bioassays for the purpose of quantitative risk assessment.[47]

Other factors should also be considered when relying upon animal bioassay results as the primary basis for quantitative risk assessments. For example, certain substances are toxic or even carcinogenic by one pathway but not by others. Nevertheless, animal bioassay protocols often emphasize the most sensitive pathway. As long as human exposure is likely to arise the same way then this choice may be reasonable. However, the pathway to which the test species is sensitive sometimes reflects an exposure route that is implausible or irrelevant for humans. For example, formaldehyde causes nasal tumors in rats at 12 times the rate observed in the next most sensitive animal species. This extreme sensitivity may be related to the fact that rats breathe only through the nose.

There may be important differences between animals and humans that make specific tumors irrelevant. For example, some chemicals cause cancer in the zymbal gland of the rat; because humans lack such a gland it is unclear whether these results matter in estimating human health risk. Other substances induce cancer through biochemical mechanisms not found in humans.

A greater controversy surrounds the question whether the same weight should be given to benign and malignant tumors. The scientific consensus is that benign and malignant tumors should be aggregated only when it is scientifically defensible to do so.[48] In practice, however, benign and malignant tumors are routinely aggregated unless a strong case can be made *against* the practice.[49] The difference between these default assumptions is significant: one approach counts only carcinomas that are present, whereas the other counts tumors that *might become* carcinomas. In an extreme case, a substance that promotes benign tumors but never causes cancer could be classified as a probable human carcinogen simply because benign and malignant tumors are treated equally.

The choice of model is actually an important policy issue.

In addition, tumor incidence is commonly pooled across sites to obtain a total estimate of carcinogenic effects.[50] This implicitly assumes that cancer induction is independent across sites and not the result of either metastasis or the same biological mechanism. Given the extreme sensitivity of test species and the regular use of MTD administration, other explanations for tumors occurring at multiple sites appear just as plausible.

The Choice of Dose-Response Model

No single mathematical model is accepted as generally superior for extrapolating from high to low doses.[51] Consequently, Federal agencies often use a variety of different models. Rather than being a scientific footnote to the risk-assessment process, however, the choice of model is actually an important policy issue. The multistage model appears to be the most commonly used method for estimating low-dose risks from chemicals, and there are two major sources of bias embedded in this choice: its inherent conservatism at low doses, and the routine use of the "linearized" form in which the 95 percent upper bound is used instead of the unbiased estimate.

The multistage model essentially involves fitting a polynomial to a data set, with the number of "stages" identified by the number of terms in the polynomial. Since animal

bioassays rarely have more than three dose levels, it is unusual to see applications of the multistage model with more than two stages. Although the multistage model enjoys some scientific support because it is compatible with multistage theories of carcinogenesis, in practice the model fails to include enough stages, due to the absence of sufficient alternative exposure cohorts.

The multistage model typically yields low-dose risk estimates that are higher than most other models. For example, when five different dose-response models were analyzed in a recent risk assessment of cadmium, estimates of cancer risks at moderate doses varied by a factor of 100. This difference among estimates widened as doses declined toward the very low levels within the range of regulatory concern. At very low doses, two of the five models predicted excess lifetime cancer risks greater than one in one thousand (10^{-3}), a risk often times regarded by policymakers as unacceptable. However, two other equally plausible models predicted essentially no excess cancer risk at all. Since none of the five models offers a scientifically superior basis for deriving low-dose risks, the choice of model is therefore a pivotal policy decision. The accepted practice under these circumstances is to develop a subjectively-derived "best" estimate while fully informing decisionmakers as to the extent of uncertainty surrounding it.[52] In the cadmium case, as in most others, this practice was not followed: Estimates of the number of statistical cancers that would be prevented by regulation were presented based only on the multistage model.[53]

The *linearized multistage model (LMS)* is a special version of the multistage model in which the 95 percent upper confidence limit of the linear term is used instead of the unbiased estimate. That is, the model identifies the largest value for the linear term that cannot be rejected at the 95 percent confidence level and uses it in place of the unbiased estimate. Assuming that the model has been correctly specified, there is only a 5 percent chance that the true risk exceeds this level.

The LMS has become the preferred statistical approach because estimates derived from it appear to be more "stable" than estimates obtained from the ordinary multistage model. The "stability" issue originally arose because unbiased estimates of low-dose risks are very sensitive to the maximum-likelihood estimate (MLE) of the value of the linear term. When the MLE of the linear term is positive, it dominates estimated risks at low doses. In some instances, however the MLE of the linear term is zero, and low-dose risk estimates decline precipitously. Using the 95 percent upper confidence limit ensures that the linear term is always positive, thus eliminating the inherent "instability' of low-dose risk estimates derived from the multistage model.[54]

Another often-cited advantage of the LMS procedure is that it provides a "yardstick" for comparing potencies across chemicals.[55] A uniform risk-assessment procedure such as the LMS, it is argued, enables policymakers to better understand the relative significance of a broad array of chemical hazards and to set regulatory priorities accordingly.

Finally, the LMS is often defended on the ground that it is prudent to err on the side of caution when dealing with potentially carcinogenic chemicals. Because the LMS generates upper-bound risk estimates, policymakers can be confident that actual risks are likely to be lower.

None of these purported advantages of the LMS approach has a sound statistical basis. It is a fundamental axiom of statistics that unbiased estimates are generally preferred to biased ones. Using the upper confidence limit instead of the unbiased estimate exaggerates underlying specification errors instead of eliminating them. "Instability" is overcome, but at the cost of greater errors in specification.

The inherent instability of the multistage model reflects a generalized misspecification of dose-response—that is, the real human dose-response relationship is often very different from what the multistage model constrains it to be. The model is extremely sensitive to small differences in observed tumor incidence, which can cause dramatic changes in estimated low-dose risks. The LMS procedure eliminates this sensitivity without remedying the underlying specification error. Proper statistical procedure requires correcting model misspecification, not masking its symptoms behind biased parameter estimates.

The LMS procedure inflates low-dose risk estimates by a factor of two or three when the MLE of the linear term is positive. However, it increases low-dose risk estimates by orders of magnitude when the MLE of the linear term is zero.[56] This means that the degree of hidden conservative bias is substantially greater for what are demonstrably lower risks.

By its very nature, the LMS cannot serve as a useful yardstick for comparing the relative risk of a variety of potential carcinogens. If a given statistical procedure generated identical biases across substances tested, then it would still yield an accurate rank-ordering of theoretical hazards. Similarly, if the procedure added a stochastic bias from a uniformly distributed random variable, the resulting rank-ordering would still be accurate on an expected-value basis. The problem with the LMS is that it generates biases that intensify with the degree to which the multistage model misspecifies the true dose-response

relationship. Even if the multistage model provided an accurate rank-ordering of hazards, the LMS could not do so, because it injects biases that are systematic with statistical misspecification.

The LMS procedure (and the multistage model itself) is also fatally flawed as a yardstick for regulatory priority setting because it fails to take account of human exposure in the calculation of unit risks. Regardless of the procedure's capacity to accurately rank-order *hazards*, failing to adjust unit risks by relative human *exposure* virtually guarantees that regulatory priorities will be misordered. Resources tend to be focused on reducing the greatest theoretical hazards rather than the most significant human health *risks*.[57]

Finally, the "margin of safety" argument in favor of the LMS unequivocally contradicts the widely recognized need to distinguish science from policy.[58] The LMS introduces into each risk assessment a conservative bias of varying but unknown magnitude. This practice fundamentally alters regulatory decisionmaking. Instead of leaving policy decisions to policymakers, the LMS disguises fundamental policy decisions concerning the appropriate margin of safety behind the veil of science.

In summary, the LMS cannot be justified as a method of scientific risk assessment. The "yardstick" defense implicitly asserts that scientific advancements in risk-assessment methodology should take a back seat to the preservation of an outdated and misguided statistical procedure. The "margin of safety" argument tacitly usurps from policymakers the authority and responsibility for risk-management decisions. Finally, the statistical "instability" overcome by the LMS is an artifact of specification error, not any scientific theory of human carcinogenesis that warrants the intentional use of biased parameter estimates. The habitual reliance upon either the multistage model or its LMS descendant cannot be supported by sound scientific principles.

Alternative models are available, of course, and they have been applied in many quantitative risk assessments. Because proper model specification is the foundation of applied statistical methodology, alternatives to the multistage model should be expected and encouraged. Indeed, innovation is the hallmark of scientific inquiry; policies that institutionalize any particular model specification effectively stifle scientific advancement.

Unfortunately, models other than the multistage model are often discouraged in practice.[59]Agencies may require substantial scientific evidence in support of an alternative model before allowing it to be used. Alternative models thus face a burden of demonstrating scientific plausibility that the multistage model cannot satisfy. Even in the extraordinary case in which this burden can be satisfied, estimates may be required from the linearized multistage model anyway.[60]

The potential human health threat posed by dioxins provides an excellent example of the problem of model selection. Using the same linearized multistage model, EPA, the Centers for Disease Control (CDC), and the Food and Drug Administration (FDA) have arrived at upper-bound risk estimates that span an order of magnitude[61]. Depending on the data and assumptions used, the linearized multistage model predicts unit risk factors that vary by as much as 1,200, with the three risk estimates mentioned earlier clustered at the high end of the range[62]. Risk assessments based on different models have led other governments to establish unit risk factors that are a thousand times less stringent than the most commonly used of these three; one study suggests that this particular estimate overstates the most likely risk estimate by a factor of almost 5,000.[63]

Conversion from Animals to Humans

Once risk has been extrapolated to low doses in rodents, scientists must convert them to human dose-equivalents. The two most common approaches involve the use of body-weight or surface-area conversions, and there are scientific reasons for choosing either approach in individual cases. The surface-area approach leads to estimates of risk that are between 7 and 12 times greater than those based on the body-weight method, depending upon the test species. Despite the ambiguity of the underlying science, the more conservative surface-area method is often applied reflexively.[64]

ISSUES ARISING FROM HUMAN EXPOSURE ESTIMATES

In addition to developing estimates of the dose-response function, agencies must estimate the likely level of human exposure. This section examines some of the issues and problems that arise in conducting an exposure assessment.

It is a generally accepted principle of exposure assessment that estimates should be based on the most likely scenario, with appropriate consideration of uncertainty.[65] Nevertheless, agencies often use conservative assumptions for exposure when real-world data are unavailable. When each of these assumptions tends to overstate likely human risks, the multiplicative effect of even a small overstatement at each stage in an exposure assessment will yield a substantial overestimate of actual exposure. For example, the multiplicative effect of overstating risk by a factor of two at five different points in an exposure assessment will overstate actual risk by a factor of 32.

Worst-Case Environmental Conditions

When data are available they often relate to unusually sensitive environments or highly contaminated conditions. When estimating regional or nationwide exposures, agencies often use data from these local "hot spots" in developing more general national estimates of health risks. However, such data are never representative and estimates extrapolated from them are generally unreliable and misleading.

In addition, chemicals often degrade naturally after they have been released to the environment. In some cases, degradation occurs very quickly, whereas in others the process may take many years or even decades. A common practice in exposure assessment modeling is to assume that exposures remain constant over time—that is, chemicals are assumed never to degrade, or degradation by-products are assumed to pose identical risks.

The Maximum-Exposed Individual

In addition to estimating the amount of a substance that may actually be present in the environment, a risk analysis must also consider the conditions under which humans may be exposed. Actual risks vary considerably depending on location, mobility, and a host of other factors. Nevertheless, estimates often are based on the upper-bound lifetime cancer risk to the maximum-exposed individual (MEI), the hypothetical person whose exposure is greater than all others. Sometimes, risks to the entire population are estimated by assuming that everyone is exposed at the MEI level. Because environmental regulations are often justified using MEI-based risk assessments, actual risks may be substantially lower than what decisionmakers and the general public perceive them to be.

In developing the MEI risk level, analyses invariably assume that the level of exposure is continuous over a 70-year lifetime. This assumption overstates actual risks, because people are mobile, encounter a constantly changing portfolio of daily risks to life and health, and can take actions that reduce risk.

Assumptions vs. Real-World Exposure Data

The thread that connects these exposure assessment issues is that simple constructs which overstate exposure are typically used in lieu of real-world data often because such data are unavailable. The risk estimates generated by these models depend on the validity of their assumptions; even small biases in exposure assessment assumptions can result in a substantial overstatement of risk.

For example, regulatory agencies may not have statistically reliable real-world data on pesticide residues in agricultural products. They also may not know the proportion of a given crop that has been treated with a particular pesticide. A common resolution of these uncertainties is to assume that residues are equal to the regulatory "tolerance—the maximum level allowed to be present in food sold in interstate commerce—and that 100 percent of the relevant crop has been treated. Both assumptions overstate actual exposure, but are encouraged by agency guidance as a way to instill conservatism in risk assessment.[66] When data are available, however, the extent of this conservative bias becomes evident. In a recent special review for the pesticide Captan, for example, EPA reduced its earlier upper-bound lifetime cancer risk estimate by two orders of magnitude when it replaced the original conservative assumptions with real-world data. Even with these improvements, EPA still reported that upper-bound risks were probably overstated. For example, field tests were performed based on applications at the maximum legal rate and as close to harvest as the label permits. Similarly, feeding studies assumed that animal diets were dominated by feedstuffs that happened to contain high residues relative to other feedstuffs, such as almond hulls and raisin waste. As EPA noted, even if these assumptions accurately represented typical animal diets, they could do so only for portions of California where these crops are grown; nationwide extrapolations based on these "hot-spots" would very likely overstate exposure.[67] Since two of the highest product-specific risks were attributed to milk and meat, these remaining conservative biases can be expected to be significant.

IMPLICATIONS OF CONSERVATIVE RISK ASSESSMENT FOR RISK MANAGEMENT AND REGULATORY DECISION MAKING

The primary purpose of risk assessment is to provide data as a basis for risk management decisions. Providing useful data requires the synthesis of information concerning risks and exposure levels into a coherent package that can be used to develop regulatory options. Decisionmakers then can use these risk estimates in evaluating regulatory alternatives. Unfortunately, the way in which risk information is characterized tends to overstate risks, making them appear much greater than they are likely to be. As a result, decisionmakers may make regulatory choices that are very different from the ones they would make if they were fully informed.

Quantification of Uncertainty

In accordance with the recommendations of the National Academy of Sciences, the OSTP Guidelines explicitly call

for the quantification of uncertainty, particularly as it arises in the selection of dose-response models and exposure assumptions.[68] Unfortunately, Federal regulatory proposals that utilize risk assessment rarely provide this information, nor do they analyze the implications of uncertainty for decisionmaking. Instead, many risk assessments only identify a lifetime upper-bound level of risk.[69]

The differences between upper-bound and expected-value estimates may be considerable. As we indicated earlier, the upper-bound risk estimate for dioxin may be 5,000 times greater than the most likely estimate. Plausible risk estimates for perchloroethylene (the primary solvent used in dry cleaning) vary by a factor of about 35,000.[70]

Risks to the entire population are estimated by assuming that everyone is exposed at the maximum level.

In some instances, decisionmakers may not be informed that risk estimates differ because of policy choices hidden in the risk-assessment methodology. In EPA's proposed rule limiting emissions from coke ovens, for example, cancer risks were estimated based on the LMS model—a model that is designed to yield upper-bound estimates of risk. In previous rules involving similar types of risks, however, EPA used the unbiased maximum likelihood estimate. To the extent that decisionmakers were not informed that the higher estimate of risk was largely due to a different low-dose extrapolation procedure, regulatory decisions based on this risk assessment were likely to reflect misunderstanding rather than science.[71]

Plausible estimates of likely cancer risk can often be found buried in regulatory background documents. However, *Federal Register* rulemaking notices seldom present such estimates alongside upper-bound estimates. This practice overstates baseline human health threats, as well as the amount of risk reduction that may be accomplished by regulation Policymakers and the public are misled because they typically see only the upper-bound estimates of the threat.

The prevalent Federal agency practice is to calculate the benefits of Federal regulatory initiatives based solely on upper-bound estimates of risk and exposure. In a recent proposal to reduce occupational exposure to cadmium, for example, the Occupational Safety and Health Administration (OSHA) developed risk estimates based on five alternative models for animal data, and two alternative models for human data. Across these seven data-model combinations, estimated excess lifetime cancer risk at the least stringent of the two proposed exposure standards varied from 0 to 153 cases per 10,000 workers occupationally exposed for 45 years. OSHA based its proposed exposure standards on one of these data/model combinations—the multistage model applied to animal data. This data/model combination predicted an excess lifetime cancer risk of 106 per 10,000 exposed workers, and was used to estimate aggregate cancer incidence and the risk-reduction benefits attributable to the new standard. Uncertainties in the underlying risk assessment, which span several orders of magnitude, were not carried forward through the exposure assessment and benefit calculation stages. This analytic error effectively obscured the uncertainty surrounding the true incidence of cadmium-induced lung cancer, and resulted in benefit estimates that may exceed actual reductions in occupational illness by several orders of magnitude.[72]

Misordered Priorities, Perverse Outcomes

Logically, one would expect that the routine overstatement of likely risks would lead to inefficient regulatory choices. Decisionmakers, convinced that a certain substance or activity poses a significant threat to public health, might well take actions that they would otherwise resist. Alternatively, they might take actions that address the wrong real-life risks.

To the extent that risk assessments differ in the degree to which they adopt conservative assumptions, it is difficult to determine which activities pose the greatest risks and hard to establish reasonable priorities for regulatory action. Because conservatism in risk assessment is especially severe with respect to carcinogens, it is reasonable to expect that other health and safety risks tend to receive relatively less attention and weight. As a result, society may actually incur greater total risk, because of misordered priorities caused by conservative biases in cancer risk assessment.[73]

A perverse and unfortunate outcome of using upper-bound estimates based on compounded conservative assumptions is that the practice may actually increase risk, even in situations where cancer is the only concern. Regulatory actions taken to address what are in fact insignificant threats may implicitly tolerate or ignore better known, documented risks that are far more serious. For

example, before it was banned, ethylene dibromide (EDB) was used as a grain and soil fumigant to combat vermin and molds. Vermin transmit disease, and molds harbor the natural and potent carcinogen aflatoxin B. The estimated human cancer risk from the aflatoxin contained in one peanut butter sandwich is about 75 times greater than a full day's dietary risk from EDB exposure. On this basis alone, it might have been appropriate to accept a small increase in cancer risk from EDB to reduce the much larger cancer risk from aflatoxin. By eliminating the relatively small hazard from EDB, Federal risk managers may have intensified the relatively potent threat of aflatoxin associated with an increase in the prevalence of mold contamination.[74]

The emphasis on risks faced by the maximum-exposed individual may also cause a perverse result by increasing overall population risks. For example, EPA's proposed regulation of the disposal of sewage sludge would probably create more public health risk than it eliminates. The proposal outlines a regulatory scheme that could shift disposal from generally safe practices to relatively risky alternatives. Thus, setting sludge quality standards to achieve an MEI upper-bound lifetime cancer risk of one in 100,000 (10^{-5}) would prevent 0.2 statistical cancer cases resulting from monofilling and land application. However, it could cause 2.0 additional statistical cancers by forcing a shift away from these disposal approaches toward incineration.[75]

These problems can be addressed by providing decisionmakers with the full range of information on the risks of a substance or an activity. Thus, decisionmakers should be given the likely risks as well as estimates of uncertainty and the outer ranges of the potential risk. Then, if regulatory decisionmakers want to choose a very cautious risk management strategy, they can do so and a margin of safety can be applied explicitly in the final decision. This approach is superior to one in which the expected risk and an unknown margin of safety are hidden behind the veil of a succession of upper-bound estimates adopted at key points in the risk-assessment process.

The public and affected parties also benefit from knowing both the expected risk and the margin of safety rather than being given upper-bound estimates that are probably very different from actual risks. People are likely to have a better intuitive understanding of the significance of averages than they have of unlikely extremes. To the extent that a margin of safety is appropriate—perhaps to protect unusually sensitive subpopulations—the magnitude of this margin can be more readily communicated if made explicit. In addition, providing information in this way should help improve public confidence in quantitative risk assessment as the basis for decisionmaking.

AVOIDING CONSERVATIVE BIASES IN RISK ASSESSMENT

Risk assessment remains a powerful and useful scientific tool for estimating many of the risks that arise in a technologically advanced society. Unfortunately, it is also susceptible to hidden biases that may undermine its scientific integrity and the basis for policymakers reliance on such information in risk management decisions. For policymakers and the public to continue to rely on risk assessment in the development of regulatory initiatives, a renewed effort must be made to separate science from policy and provide risk information that is both meaningful and reliable.

Expected Value Estimates

Perhaps the most important current need in regulatory decisionmaking is for carefully prepared and scientifically credible estimates of the likely risks involved. Relying on worst-case analysis based on extremely conservative risk assessment and exposure models leads to widespread misunderstanding on the part of both Government officials and individual citizens. Decisionmakers at all levels need unbiased and impartial risk information so they can focus their attention on significant problems and avoid being distracted by minutiae.[76]

Weight-of-Evidence Determinations

Similar procedures are needed for assigning weights to each relevant study in the risk-assessment literature. Current practice gives undue weight to studies that show positive relationships. Resulting risk classifications are thus conservatively biased estimates derived from samples of similarly biased observations.

Full Disclosure

Efficient and responsible decisionmaking requires that policymakers and the public be fully informed about the implications of the regulatory alternatives among which they must choose. Meeting this requirement demands a careful discrimination between science and policy. When risk estimates depend on assumptions and judgments instead of data, the meaning and implications of these nonscientific parameters must be clearly articulated.

Avoiding Perverse Outcomes

Careful attention needs to be paid to the likely results of regulatory alternatives, with an eye toward avoiding choices that have the perverse effect of increasing net risk. All human activity involves risk.

Decisionmakers need to be sure that specific actions taken in the name of risk-reduction in one area do not make

matters worse elsewhere. Quantitative risk assessment can help in this regard so long as the methods applied are not inherently biased in a way that undermines comparisons across alternatives, each of which entails some degree of risk.

Our discussion has covered only the highlights of risk-assessment methods, yet we have identified several independent places at which conservative assumptions are commonly used. Individually, each of these assumptions might appear to be prudent responses to scientific uncertainty. In combination, however, they result in a distortion equal to the product of the individual conservative biases. To illustrate, suppose that there are ten independent steps in a risk assessment and prudence dictates assumptions that in each instance result in risk estimates two times the expected value. Such a process would yield a summary risk estimate that is more than 1,000 times higher than the most likely risk estimate. Because there are usually many more than ten steps, and many of them will incorporate conservative biases that exceed an order of magnitude, risk estimates based on such practices will often exceed the most likely value by a factor of one million or more.

When risk assessments contain hidden value judgments, their scientific credibility is inevitably compromised. To the extent that policymakers and the public fail to understand the magnitude of the margin of safety embedded in quantitative risk assessments; policy choices are distorted from the course that would have been selected if decisionmakers had been better informed of the actual risks. Ironically, these policy decisions may actually increase total societal risk. Too much attention is focused on relatively small hazards that have been exaggerated by conservative risk assessments, leaving alone larger risks that have been estimated using unbiased procedures.

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[20] Researchers, using different methods, assumptions, and time periods, have formed incomplete estimates by adding up the cost of individual regulations. These estimates accordingly show considerable variation for current annual costs ranging from $60 billion to $175 billion a year-5 to 15 percent of current Federal outlays.

[21] National Academy of Sciences, *Risk Assessment in the Federal Government: Managing the Process,* Washington, DC: National Academy Press. 1983 (hereinafter, *NAS Risk Management Study),* p.151.

[22] *Ibid.,* p 153.

[23] For representative views of risk-assessment practitioners see, e.g., Lester B. Lave, *The Strategy of Social Regulation: Decision Frameworks for Policy,* Washington, DC: Brookings, 1981; Lester B. Lave, "Methods of Risk Assessment," Chapter 2 in *Quantitative Risk Assessment in Regulation,* Lester B. Lave, ed., Washington, DC: Brookings, 1982, esp. pp. 52-54. For representative views of risk-management officials see, e.g., William D. Ruckelshaus, "Science, Risk, and Public Policy," *Vital Speeches of the Day,* Volume 49, No. 20, August 1, 1983, pp. 612-615.

[24] See, *e.g.* Howard Kunreuther and Lisa Bendixen, "Benefits Assessment for Regulatory Problems," and Baruch Fischhoff and Louis Anthony Cox. Jr., "Conceptual Framework for Regulatory Benefits Assessment," Chapters 3 and 4, respectively, in *Benefits Assessment: The State of the Art,* Judith D. Bentkover, Vincent T. Covello, and Jeryl Mumpower, eds., Dordrecht, Netherlands: D. Reidel, 1986, pp. 44-45, 50-61

[25] See U.S. Office of Science and Technology Policy, "Chemical Carcinogens: A Review of the Science and Its Associated Principles", Principle 29 (50 FR 10378. March 14, 1985, hereinafter, *OSTP Risk Assessment Guidelines);* US Environmental Protection Agency, "Guidelines for Carcinogenic Risk Assessment," 51 FR 34001 (September 24, 1986, hereinafter, *EPA Carcinogen Risk Assessment Guidelines);* US Department of Health and Human Services, *Risk Assessment and Risk Management of Toxic Substances,* April 1985, p.20.

[26] William D. Ruckelshaus, *(op.cit.),* p.614. *OSTP Guidelines,* Guideline 8, p. 10376.

[29] See, *e.g.,* Bruce Ames, Renae Magaw and Lois Swirsky Gold, "Ranking Possible Carcinogenic Hazards", *Science,* Vol, 236, April 17, 1987; Gio Batta Gori, "The Regulation of Carcinogenic Hazards," *Science,* Vol 208, April 18, 1980.

[29] *OSTP Guidelines,* Guideline 11, p. 10377.

[30] In the original analysis of the rat bioassay used to derive the dose-response function for dioxin, 9 of 85 controls were said to develop liver tumors. An independent review of this data resulted in 16 of the 85 controls being classified as having such tumors. See US Environmental Protection Agency, *A Cancer Risk-Specific Dose Estimate for 2,3, 7, 8-TCDD, Appendix A,* EPA/ 600/6- 88/007Ab, June 1988 (hereinafter, *Dioxin Risk Assessment Appendix A),* pp. 2-3.

[31] Colin N. Park and Ronald D. Snee, "Quantitative Risk Assessment: State-of- the-Art for Carcinogenesis," Chapter 4 in *Risk Management of Existing Chemicals,* Rockville, MD Government Institutes, 1983, p. 56.

[32] Alvan R. Feinstein, Scientific Standards in Epidemiological Studies of the Menace of Daily Life," *Science,* Vol. 242, December 2, 1988. pp. 1257-1263.

[33] Linda C. Mayes, Ralph I. Horowitz, and Alvan R. Feinstein, "A Collection of 56 Topics with Contradictory Results in Case-Control Research," *International Journal of Epidemiology,* Vol. 17, No. 3 (1988), pp. 680-685.

[34] Ames *et al., (op. cit.),* p. 276.

[35] *Dioxin Risk Assessment Appendix A,* pp. 2-3.

[36] See Ames *et al., (op. cit.,* p. 276 (arguing that such data are irrevelant); *OSTP Guidelines Guideline* 9, p. 10377 (concluding that such data "must be approached carefully"); and *EPA Carcinogen Risk Assessment Guidelines,* p. 33995 (making the policy judgment that such data are sufficient evidence of carcinogenesis). Liver tumors dominated in EPA's dioxin risk assessment. See *Dioxin Risk Assessment, appendix A,* pp.2-3.

[37] See *OSTP Guidelines,* Guideline 25, p. 10378; *EPA Carcinogen Risk Assessment Guidelines,* p. 33995.

[38] See *EPA Carcinogen Risk Assessment Guidelines,* p. 3399934000. A single animal test that shows a positive result "to an unusual degree" (p. 33999) is sufficient to warrant at least a B2 classification ("probable human carcinogen"), even if this result occurs in a species known to have a high rate of spontaneous tumors. A strong animal bioassay or epidemiological study showing no evidence of carcinogenic effect cannot overcome this presumption (p. 34000).

[39] See "Second Peer Review of Daminozide (Alar) and UDMH (Unsymmetrical 1,1-dimethylhydrazine)," Memorandum from John A. Quest to Mark Boodee, U.S. Environmental Protection Agency, OPTS, May 15, 1989 (hereinafter, *Alar/UDMH Internal Peer Review No.* 2). This internal OPTS panel reviewed several recent studies on Alar and UDMH.

One study of Alar yielded a statistically significant increase in common lung tumors in mice, but only for one of three dosage levels. Results were not statistically significant at one higher and two lower dosages, and controls also displayed unusually high tumor incidence. 90% of the lung tumors in dosed mice were benign, versus 89% in the controls.

One study of UDMH yielded statistically significant increases in common lung and uncommon liver tumors in mice, but only for the higher of two dosages. 97% of the lung tumors in dosed mice were benign, versus 100% in the controls. 29% of the liver tumors in dosed mice were benign; no tumors were observed in the controls. Prior studies that purported to show a carcinogenic response had been judged inadequate by EPA's Scientific Advisory Panel, an external peer review group. The Office of Pesticides and Toxic Substances (OPTS) panel noted that a different internal EPA risk-assessment panel (the Carcinogen Assessment Group) considered these studies sufficient to justify B2 classifications when it evaluated them for EPA's Office of Solid Waste and Emergency Response. Despite the scientific controversy, the OPTS panel interpreted these prior studies as "supporting evidence" under EPA's risk-assessment guidelines.

[40] See *EPA Carcinogen Risk Assessment Guidelines,* p. 33995 (establishing the need for replicate identical studies showing no effect), and p. 33999 (establishing the minimum requirement of two well-designed studies showing no increased tumor incidence to warrant a "no evidence" determination).

[41] *Alar/UDMH Internal Peer Review* No. 2, pp. 6, 8, 9. EPA's scheme for carcinogen classification is itself an issue among scientists. See, *e.g.,* US Environmental Protection Agency, Risk Assessment Forum, *Workshop Report on EPA Guidelines for Carcinogen Risk Assessment,* EPA/625/3-89/015, Washington. DC: March 1989, pp. 21-26.

[42] See *EPA Carcinogen Risk Assessment Guidelines,* p. 33997 (data from long-term animal studies showing the greatest sensitivity should generally be given the greatest emphasis.

[43] See, *e.g.,* Ames *et al., (op. cit.),* pp. 276-277.

[44] *OSTP Guidelines,* Guideline 25, p. 10378; *EPA Carcinogen Risk Assessment Guidelines,* p. 34003 (responding to comments on the draft guidelines and affirming agreement with OSTP Guideline 25).

[45] Lester B. Lave, Fanny K. Ennever, Herbert S. Rosenkranz, and Gilbert S. Omenn, "Information Value of the Rodent Bioassay," *Nature,* Vol. 336 (December 15, 1988), pp. 631-633.

[46] *False negatives* occur when a test fails to detect effects when they are in fact present. *Sensitivity* refers to the capacity of a test to minimize false negatives. *False positives* occur when a test appears to detect effects that in fact are absent. *Selectivity* refers to a test's ability to minimize false positives. The 9 to 1 ratio of false positives to false negatives calculated by Lave *et al.* assumes that both selectivity and sensitivity equal about 70%.

[47] Lave *et al., (op. cit.),* p. 631. Adjusting also for less sensitivity reduces the ratio of false positives to false negatives. For example, if sensitivity is only 10 percent and all other parameters remain unchanged, then this ratio declines to 9.5 to 1. However, this implies that both types of statistical errors are rampant, which raises questions concerning the practical utility of animal bioassays This is, in fact, precisely the concern raised by Lave *et al., (op. cit.),* who conclude that such tests are cost-effective investments in information only under extraordinary conditions.

[48] *OSTP Guidelines,* p. 10376.

[49] *EPA Carcinogen Risk Assessment Guidelines,* p. 33997.

[50] *Id.*

[51] *OSTP Guidelines,* Guideline 26, p. 10378; Ames *et al., (op.* cit.), p. 276.

[52] See, *e.g., OSTP Guidelines,* Guidelines 27, 29, and 31, p. 10378; *EPA Carcinogen Risk Assessment Guidelines,* pp. 33999, 34003.

[53] Occupational Safety and Health Administration, "Occupational Exposure to Cadmium; Proposed Rule," 55 FR 4076 (February 6, 1990).

[54] Albert L. Nichols and Richard J. Zeckhauser, "The Dangers of Caution: Conservatism in Assessment and the Mismanagement of Risk," Chapter 3 in *Advances in Applied Micro-Economics, Volume 4: Risk, Uncertainty, and the Valuation of Benefits and Costs,* V. Kerry Smith, ed., Greenwich, CT: JAI Press, 1986, pp. 55-82, esp. pp. 62-63. A nontechnical version of this paper is available by the same authors as "The Perils of Prudence: How Conservative Risk Assessments Distort Regulation," *Regulation,* November/December 1986, pp. 13-24.

[55] US Environmental Protection Agency, *A Cancer Risk-Specific Dose Estimate for 2,3, 7,8-TCDD,* EPA/600/6-88/007Aa, June 1988 (hereinafter, *Dioxin Risk Assessment),* pp. 45-46.

[56] Nichols and Zeckhauser, *op cit.,* pp. 62-63.

[57] Some scientists have attempted to devise alternative indexes of relative human health risk that explicitly account for variations in human exposure. Ames *et al., (op. cit.),* pp. 272-273, describe one such alternative (the Human Exposure/Rodent Potency index, or HERP) and report index values for 36 substances. Because the HERP index is based on a relative rather than absolute scale, the distorting effect of conservative biases embedded in the underlying risk assessments has been significantly reduced. Many substances suspected of being environmental carcinogens rank very low on the HERP index, suggesting that regulatory priorities have been seriously misdirected.

[58] See *e.g., NAS Risk Management Study,* p. 161; *OSTP Risk Assessment Guidelines,* Principle 29, p. 10378; and *EPA Carcinogen Risk Assessment Guidelines,* p. 34001.

[59] See, *e.g.,* Ames *et al., (op. cit.),* p. 276 (continued reliance on linear models despite the accumulation of evidence against linearity); and Lester B. Leve, "Health and Safety Risk Analysis: Information for Better Decisions," *Science,* Vol. 236, April 17, 1987, pp. 291-295, esp. p. 292 (agencies often resist modeling improvements and data that yield lower risk estimates).

[60] *EPA Carcinogen Risk Assessment Guidelines,* pp. 33997-33998. "In the absence of adequate information to the contrary, the linearized multistage procedure will be employed.... Considerable uncertainty will remain concerning responses at low doses; therefore, in most cases, an upper-limit risk estimate using the linearized multistage procedure should also be presented."

[61] *Dioxin Risk Assessment Appendix A,* p. 13. Unbiased risk estimates vary by a similar factor.

[62] *Dioxin Risk Assessment,* pp. 46-49.10^{-6} risk-specific doses (RsDs) derived from the linearized multistage model span the range from 0.001 to 1.2 picogram/kg/day. The RsDs of EPA, CDC, and FDA are 0.006, 0.03, and 0.06 pg/kg/day, respectively.

[63] *Dioxin Risk Assessment,* p. 4.

[64] *EPA CarCinogen Assessment Guidelines,* p. 33998. "EPA will continue to use this [surface area] scaling factor unless data on a specific agent suggest that a different scaling factor is justified."

[65] EPA guidance documents have historically called for unbiased estimates of exposure. See, *e.g.,* US Environmental Protection Agency, "Guidelines for Exposure Assessment," 50 FR 34042-34054 (September 24, 1986, hereinafter, *EPA Exposure Assessment Guidelines);* US Environmental Protection Agency, *Superfund Public Health Evaluation Manual,* OSWER Directive 9285.4-1, October 1986; and US Environmental Protection Agency, *Superfund Exposure Assessment Manual* (Revised Draft), OSWER Directive 9285.5-1, December 1986. EPA recently abandoned the calculation of unbiased exposure estimates for Superfund sites on the ground that it was insufficiently conservative. EPA's new protocol requires the estimation of "reasonable maximum exposure" instead of the average and upper-bound estimates. Reasonable maximum exposure constitutes a new term of art that EPA intends to be "well above the average case" but not as extreme as the upper-bound. It provides a new opportunity for embedding conservative assumptions into exposure assessment and exaggerating estimates of actual human-health risk at Superfund sites. See *Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A), Interim Final,* EPA/540/1-89/002, December 1989, Chapter 6. pp. 5, 47-50.

[66] *EPA Exposure Assessment Guidelines,* p. 34053. "When there is uncertainty in the scientific facts, it is Agency policy to err on the side of public safety."

[67] See, *e.g.,* US Environmental Protection Agency, "Captan: Intent to Cancel Registrations; Conclusion of Special Review," 54 FR 8127-8128 (February 24, 1989).

[68] *OSTP Guidelines,* (Guideline 27), p. 10378.

[69] See, *e.g., EPA Carcinogen Risk Assessment Guidelines,* p. 33998.

[70] Nichols and Zeckhauser, *(op. cit.),* pp. 64-65.

[71] Letter from Wendy Gramm (Administrator of the Office of Information and Regulatory Affairs) to Lee Thomas (Administrator of the Environmental Protection Agency), August 12, 1986, p. 3.

[72] Occupational Safety and Health Administration, "Occupational Exposure to Cadmium; Proposed Rule," 55 *Federal Register* 4076, 4080, 4093.

[73] This is precisely the policy issue raised by Nichols and Zeckhauser, *(op. cit.),* pp. 69-71, who note that EPA's 1985 decision to limit lead in gasoline was threatened by concerns about potential increases in benzene exposure. Any tradeoff between lead and benzene risks would have been biased against lead; as estimates of benzene risks are more conservative simply because it is a carcinogen, whereas lead is not.

[74] Ames *et al., (op. cit.),* p. 273.

[75] US Environmental Protection Agency, "Standards for the Disposal of Sewage Sludge; Proposed Rule," 54 FR 5746-5902 (February 6, 1989).

[76] Nichols and Zeckhauser, *op. cit.,* pp. 72-76.

Styrene and its Metabolites: A Discussion of Results from Cytogenetic Assays

R. Julian Preston, Ph.D.
Section Head, Biology Laboratory
Oak Ridge National Laboratory

A wide variety of different assay systems have been utilized to determine the possible effects of chemical agents on alterations of chromosome structure and number. The endpoints that are analyzed are chromosomal aberrations (deletions of parts of chromosomes or rearrangements of chromosomes), sister chromatid exchanges (apparently reciprocal exchanges between the two chromatids of a single chromosome) and aneuploidy (gains or losses of one or more chromosomes). The cell types studied in these assays are most frequently:

In vivo treatments:	Bone marrow cells (rodent)
	Peripheral lymphocytes (human and rodent)
	Spermatogonia and spermatocytes (rodent)
In vitro treatments:	Chinese hamster cell lines
	Peripheral lymphocytes (human)

Whilst these different assays can provide important information on the induction of chromosomal alterations by chemical and physical agents, and in some specific cases can provide information on exposure range, none can be used directly to estimate the adverse health effects (somatic or genetic) that might arise from exposure to a particular agent. The reasons for this include: unsuitability of cell type for direct extrapolation; end-point does not have known biological consequences (sister chromatic exchange [SCE]); specific alterations that are or might be related to genetic or somatic adverse effects are not measured. Thus, genotoxicity results should, in the majority of cases, be restricted in interpretation and be considered within the context of the assay, *i.e.* can an agent induce cytogenetic effects under the particular conditions of the assay.

A possible association between styrene exposure and chromosomal damage in humans has been the subject of numerous investigations in recent years, particularly in Europe. While these studies have been largely inconclusive, conclusions have sometimes been drawn from them that give rise to considerable confusion and concern outside the scientific community. In this article, Dr. Preston examines the principal assays for chromosomal analysis and discusses their use for genotoxicity assessment in cellular, animal and human studies. He notes that the better conducted human monitoring studies indicate no difference in aberrations between those exposed to styrene and the control groups who were not exposed.

There are many protocols published for each assay, but those that are acceptable have taken into account certain principles that will maximize the sensitivity of the assay, and decrease the probability of obtaining false negative results. These principles include: analyzing cells for chromosome aberrations in their first mitotic division after treatment; sampling cells at a time when the majority of metaphases are cells that were treated during the DNA synthesis phase; for SCE analysis using sampling times for control and treatment groups when similar proportions of 2nd division cells are present; selecting numbers of analyzed cells and/or numbers of individuals that allow for adequate statistical analysis, and for human monitoring reduce the influence of confounding factors that can affect sensitivity. A discussion of these facts and a description of appropriate assays can be found in Preston *et al.* 1981; Latt *et al.* 1981; Preston *et al.* 1987a; Preston *et al.* 1987b.

The published literature on the induction of chromosome aberrations and SCE

by styrene and styrene-7,8-oxide (the primary intermediate, see Fig. 1) will be discussed in this report with regard to the acceptability of the assay based on the principles described above, and how this might influence the interpretation of the results.

INDUCTION OF CHROMOSOME ABERRATIONS AND SISTER CHROMATID EXCHANGES BY STYRENE AND STYRENE OXIDE

1. In vitro treatment

a. Chinese hamster cells

(i) Chromosome aberrations

There is a very small amount of literature on the clastogenic effects of styrene and styrene oxide in cultured Chinese hamster cells, and, in fact, the data are of only limited value.

Matsuoka *et al.* (1979) have utilized a standard protocol to study the induction of chromosome aberrations in Chinese hamster lung cells by a wide range of chemical agents. Styrene monomer was tested at a single concentration (0.25 mg /ml, 2.4 mM) with and without metabolic activation (S9 fraction from 3-methylcholanthrene induced rat livers) and were sampled 24 h after a 3 h treatment. This is not an appropriate protocol: only one concentration; a high proportion of mitotic cells were probably in their 2nd division after treatment; sampled population not necessarily of cells treated in the DNA synthesis phase. The results (presented as a single line in a table) show that there was no increase in chromosome aberrations in the absence of S9, and a stated increase with S9. However, the category of aberrations includes gaps as well as deletions and exchanges, and the frequencies of each class cannot be deducted. Gaps (or achromatic lesions) are not considered to be true aberrations and their significance is unknown (Preston *et* al. 198713). They should be reported separately from aberrations. Thus these data cannot be used to determine the potential clastogenicity of styrene monomer or its metabolites.

Turchi *et al.* (1981) studied the cytogenetic effects of styrene oxide in Chinese hamster V-79 cells. A single concentration (0.75 mM) was used with sampling times of 25, 40 and 50 h following a 1 h treatment. The end-points were anaphase bridges and micronuclei—a rather poor choice for assessing clastogenic effects. The assay protocol is inadequate because of the sampling times and the use of single concentration. These inadequacies do not necessarily negate a positive response, and it can be concluded from the data that the frequency of micronuclei and anaphase bridges are increased by 0.75 mM of styrene oxide. In addition it was demonstrated that styrene oxide was an effective alkylator of 4-(p-nitrobenzyl)pyridine, indicating its potential mode of action for inducing chromosomal effects.

(ii) Sister chromatid exchanges

de Raat (1978) studied the induction of SCE in Chinese hamster ovary cells by styrene and styrene oxide. Styrene oxide was an effective inducer of SCE over the concentration range of 25-100 μl/l in the absence of S9. In the presence of S9 (from phenobarbital induced rat liver) there was no increase in SCE indicating a conversion of styrene oxide to non-active metabolites (See Fig. 1). There is an increase in SCE in the presence of S9 when cyclohexene oxide (0.1 ml/l) is used as an inhibitor of epoxide hydratase that would normally metabolize styrene oxide, and is present in rat liver S9. Styrene in the absence or presence of S9 over a concentration range of 10-100 μl/l does not induce an increase in SCE. There is an increase when cells were treated with 500 or 1000 μl/l styrene in the presence of S9 and cyclohexene oxide. These results suggest that styrene can cause SCE, but only when metabolized and when epoxide hydratase activity is inhibited. The effects of *in vivo* exposure to styrene cannot be reliably predicted from these data.

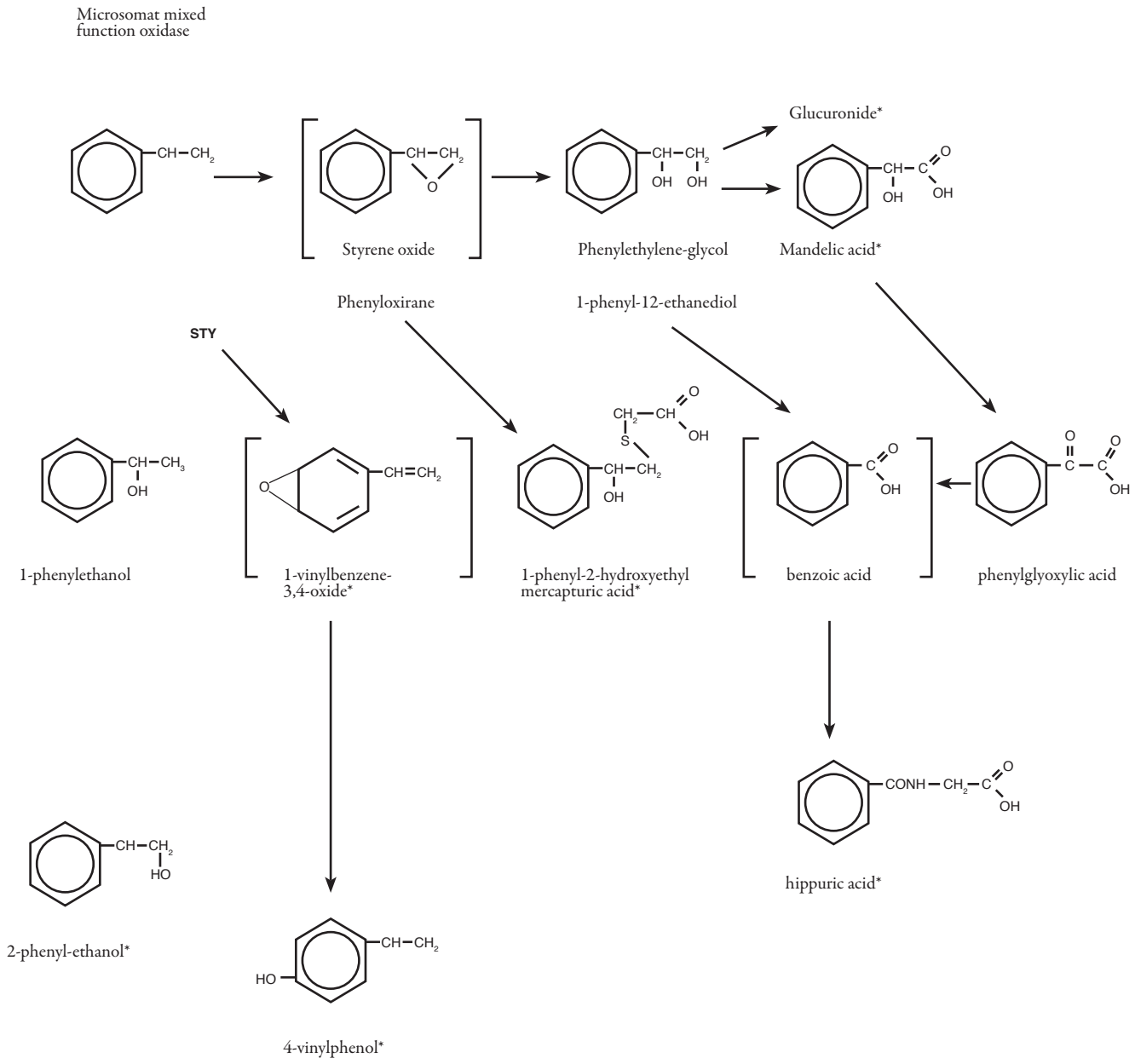
b. Human lymphocytes

(i) Chromosome aberrations

There are five original reports on the treatment of human lymphocytes *in vitro* with styrene and styrene oxide. The major problem with these studies is that in all cases only one blood donor was used. This does not necessarily negate the results with regard to the clastogenicity of styrene or its metabolites, but it does not allow for a consideration of differences in individual response, particularly for possible differences in metabolism.

Linnainmaa *et al.* (1978) analyzed the induction of chromosome aberrations by styrene and styrene oxide in lymphocyte cultures from a single donor. A single concentration of styrene (0.03% v/v) was added to the cultures throughout the 72 h culture period. Styrene oxide was also used at a single concentration of 0.008% v/v but for the last 8 h of culture because of its toxicity. The sampling time of 72 h after the addition of the mitogen is not generally acceptable because in the absence of significant cell cycle delay a high proportion of metaphases will be in their 2nd or 3rd *in vitro* divisions. However, this will reduce the sensitivity of the assay, and will not negate the observation of a positive response. Styrene was reported to increase the frequency of "breaks" compared to the control, but it cannot be determined if gaps were included in this category. The mitotic index was reduced following treatment with

FIGURE 1 SCHEMATIC OF METABOLISM AND REACTIONS OF STYRENE



* Excreted in urine

styrene indicating that there was a measurable effect upon the cells.

Styrene oxide appears to be more effective than styrene at inducing a cytogenetic response since at a lower concentration it produced pulverized chromosomes. The frequency of aberration was only slightly increased, and again it is not clear whether or not gaps were included. Styrene oxide caused a significant decrease in mitotic index. (Styrene glycol, a metabolite of styrene oxide, did not affect mitotix index). The tissue culture medium can influence the response in some specific cases but the one used in these studies was not given.

Fabry *et al.* (1978) carried out a study of the effects of styrene oxide on chromosome aberration frequencies in human lymphocytes *in vitro,* and also in mouse bone marrow and germ cells following *in vivo* exposures [reported in Section 2a(i)]. One donor was used, lymphocytes were cultured in Ham's Fl0 medium for 72 h before fixation, and styrene oxide was added 24 h after culture initiation at concentrations of 0.1 and 0.5 mM (1 mM was markedly toxic). A small increase in chromosome aberrations was reported at both concentrations of styrene oxide compared to the control. It should be noted that twice as many cells (250) were analyzed in the treatment groups, and so the aberration values per 100 cells (a common method of reporting a low frequency event) are 1/2 the values shown in the authors' Table 1. In fact, the aberration frequencies following exposure to styrene oxide are about the same as those frequently reported for background frequency in the general population. The assay is not ideal because of the 72 h fixation, and because only two concentrations were used. There was no increase in aberrations, dominant lethal mutations, or micronuclei in any of the *in vivo* assays [discussed in Section 2a(i)].

Norppa *et al.* (1981) also studied the induction of chromosome aberrations by styrene-7,8-oxide (S.O.) in human lymphocytes *in vitro.* They also used only one donor, but with 3 treatment concentrations 6.0μg/m1 (0.05 mM), 24.0 ml and 48.0 μg/ml. The cultures were treated with S.O. for 24 h, from 24 h after adding the mitogen until the cultures were fixed at 48 h, or 48 h for cultures fixed at 72 h. In the latter case bromodeoxyuridine (BrdU) was added to the cultures so that the chromosome preparations could be differentially stained (as for SCE) and 1st division mitoses only could be analyzed.

The tissue culture medium used was TC 199[1]. The frequency of aberrations was not increased following the 6 μg/m1 treatment but was increased (for chromatid breaks) at 24 μg/ml. No metaphases could be analyzed after the treatment with 48 μg/ml. When only 1st mitotic cells were analyzed from the 72 h cultures a four-fold greater effect was seen at 24 μg/ ml, and significant increases in chroma tid exchanges were also seen. This not only shows that styrene oxide was clastogenic, but also emphasizes the importance of analyzing metaphases in their 1st division after treatment.

Pohlova *et al.* (1985) studied the effects of styrene and styrene oxide on the induction of chromosome aberrations in human lymphocytes *in vitro,* and also whether there were interactive or additive effects between styrene or S.O. and THIO-TEPA (an alkylating agent showing potent genotoxicity). The cells were treated with styrene (5×10^{-4}, 1×10^{-4}, 1×10^{-5}, or 5×10^{-6} mol/1) and S.O. (1×10^{-3}, 5×10^{-4}, 9×10^{-5}, 6×10^{-5}, 3×10^{-5} or 5×10^{-6} mol/1) for the last 24 h of culture, and the cultures were fixed at 54 h after mitogenic stimulation. The tissue culture medium used was not recorded. It is difficult to adequately assess the results because they are only presented graphically as total aberrations. Styrene appears to only induce an increase in aberrations at the higher concentration of 5×10^{-4} mol/1, and even at this concentration the effect is very small. The frequencies with styrene plus THIO-TEPA were the same as with THIO-TEPA alone. Styrene oxide produced a significant increase in aberrations at 5×10^{-4} mo1/1 with marginal to small increases at lower concentrations. Styrene oxide and THIO-TEPA in combination produced additive effects. The clear-cut dose response described by the authors is not apparent from the data as presented and no interpretation can be made without the benefits of their statistical analysis.

(ii) Sister chromatid exchanges

In two of the studies reported above for chromosome aberrations, SCE were also analyzed, and appeared to be more conclusive than the aberration studies. A third study reported only SCE data.

Norppa *et* al. (1980a) analyzed SCE in human lymphocytes treated *in vitro* with styrene or styrene oxide, and also measured the levels of S.O. in styrene treated cultures by gas chromatography. Styrene (0.33-4.0 mM) and styrene oxide (0.07, 0.14 or 0.33 mM) were added 24 h after establishing the cultures, and remained until fixation of the cells 48 h later. There was a linear increase in SCE for both styrene and S.O. up to 50 SCE per cell at the highest concentration of each. Styrene oxide was about 10 x as effective as styrene.

The gas chromatographic data was obtained from cultures treated with 52 lmol of styrene 24 h after mitogenic stimulation, for 30 min, 2 h, 6 h, or 24 h. The levels of S.O. were also measured in cultures treated with 4.4 lmol. The styrene to S.O. ratios progressively increased with incubation time in styrene-treated cultures. The amount of S.O.

decreased with incubation time in S.O.-treated cultures. These data suggest that styrene and S.O. can both be metabolized by human whole blood cultures—the cells involved in this process were not identified.

Norppa *et al.* (1981) also demonstrated an increase in SCE following treatment with S.O., but from their Table 3, it is stated that the data were in part previously published in Norppa *et al.* (1980)—discussed above. It appears that they are the very same data and need no further discussion.

Norppa and Vainio (1983) studied the induction of SCE in the lymphocytes of a single donor by styrene and eleven styrene analogs. The medium used was TC199, and although its use is not recommended for chromosome aberration studies, it is not known to affect SCE frequencies. The treatment time was 48 h—from 24 h after mitogenic initiation to fixation at 72 h. There was an increase in SCE in cells treated with styrene, analogs substituted with methyl groups at the 2-; 3-; 4-; 3,5- or ß-positions, and 3,5- dimethylstyrene-7,8-oxide and 4-nitrostyrene-7,8-oxide. The increases were generally dose dependent for the styrene-7,8-oxides over a range of 0.1 to 0.3 mM and for styrene and the methyl styrenes over a range of 1 to 3 mM. 4-methylstyrene-7,8-oxide and a-methylstyrene induced a marginal or no increase, respectively. Ethylbenzene and 2-phenylethanol (styrene analogs without a double bond in the side-chain) were ineffective. In general, the vinyl group appears to be important in the activation of styrene-like compounds, and the products of this activation would be styrene-7,8-oxides. It can be concluded that whole blood cultures can metabolize styrene and its analogs to styrene oxides, although the cells performing this activation could not be determined.

Norppa *et al.* (1983) also studied the induction of SCE in human lymphocyte cultures by styrene and styrene-7,8oxide. The styrene and styrene oxide were added to the cultures 24 h after mitogen initiation and remained until fixation at 72 h; 11 donors were used (6 were workers from the reinforced plastics industry and 5 were research personnel) and the treatment concentrations were 2 mM for styrene and 0.15 mM for styrene oxide. These treatment concentrations both induced about a 3-fold increase in SCE compared to untreated controls. Thus styrene-7,8-oxide was about 10 times as effective as styrene. They also demonstrated that purified lymphocytes cultured in the presence of small numbers of erythrocytes (about 20,000 per ml, compared to 200-400 million/ ml in whole blood cultures) showed no increase in SCE following treatment with styrene over the concentration range of 0.5-4 mM. It appears that erythrocytes are necessary to metabolically activate styrene, probably to the 7,8-oxide. It is interesting to note that there was no difference in SCE frequencies among the individuals in the "control" group although 6 were potentially exposed to styrene since they were reinforced plastic workers. Also there was no difference in response among the donors when lymphocytes were treated with styrene or styrene oxide. Although the choice of donors is rather unusual the results and conclusions appear to be valid. The same data are reported in Norppa *et al.* (1984).

Pohlova *et al.* (1985) measured SCE frequencies in human lymphocytes *in vitro* treated with S.O. at concentrations of 1×10^{-3}, 2×10^{-4}, 9×10^{-5}, 6×10^{-5}, 3×10^{-5}, 1×10^{-5}, or 5×10^{-6} mo1/1. Treatment in the presence of BrdU was from 48 h after mitogenic stimulation until fixation 48 h later. There was a linear increase in SCE over the concentration range, up to approximately 30 SCE/cell at 2×10^{-4} mol/1.

CONCLUSIONS FROM *IN VITRO* STUDIES

Styrene appears to be marginally or non-effective at inducing aberrations in rodent and human cells, whereas styrene oxide is clastogenic in human lymphocytes. Styrene and S.O. can induce SCE in human lymphocytes, but not in Chinese hamster cells (based on one study). The reasons for differences between species cannot be determined from the studies, but might represent differences in metabolic activation of both styrene and S.O.

2. In vivo *exposures*

a. Rodents

(i) Chromosome aberrations

Loprieno *et al.* (1978) conducted a fairly extensive study of the effects of styrene and styrene oxide in point mutation, chromosome aberration and DNA repair assays. The induction of aberrations in mouse bone marrow cells will be discussed here. Mice (male and female, 10-14 week old, CD-1) were treated by gavage (single dose, 66% in olive oil) with styrene (500, 1000 mg/kg body weight) and styrene oxide (50, 500, 1000 mg/kg) and bone marrow cells sampled 24 h later. Bone marrow cells are cycling, and in order to meet the criteria of the protocols for aberration assays *(i.e.,* sampling cells at their 1st mitosis post-treatment and analyzing cells that were in the DNA synthesis phase whilst the agent or its metabolites were active) more than one sampling time should be used, particularly one at an earlier time than 24 h. Failure to do this will frequently reduce the sensitivity of the assay. Styrene did not induce an increase in aberrations at either concentration. Styrene oxide induced a small increase in chromatid deletions at 500 and 1000 mg/kg, but not at 50 mg/kg. The large increase reported by the authors includes gaps (not considered true

aberrations) and a category called "Others" that is not defined, but is about 3% in the controls. For the categories that are generally included in aberration assays, only a small increase appears to be induced by S.O.

Fabry *et al.* (1978) conducted a similar study, but used a single i.p. injection of 250 mg/kg body weight styrene oxide, and analyzed bone marrow cells (BALB/c male mice) at 1, 2, 6, or 13 days after treatment. As above, those sampling times are not suitable for the analysis of primarily 1st division cells, and at the later times only chromosome aberrations. Only one chromatid deletion was seen (on day 2 after treatment) and although no control data are given, styrene oxide clearly was negative in this assay. However, the assay is not adequate to draw any firm conclusion.

The same authors also studied the induction of micronuclei in mouse polychromatic erythrocytes following 250 mg/kg i.p. treatment with styrene, the induction of dominant lethals in post-meiotic male germ cells, and the induction of translocations in spermatogonial stem cells analyzed in primary spermatocytes. The methods used in these assays are open to criticism, and the report of negative responses should be considered to be far from unequivocal.

Meretoja *et al.* (1987) analyzed rat bone marrow cells after inhalation of styrene. Exposures were to 300 ppm for 2-11 weeks (6 h per day, 5 days per week), with animals being sampled weekly, with the majority of animals in the 7 to 11 week groups. No increase in aberration frequency were observed in the styrene-treated group compared to the control during the first 8 weeks of exposure. At longer exposure times the authors report an increase in aberrations. However, they state that most of the aberrations are "chromosome-type breaks". This result is difficult to understand. The vast majority of chemical agents, including all alkylating agents, induce aberrations in cells in the DNA synthesis phase or cells that pass through this phase with unrepaired DNA damage—the aberrations are of the chromatid-type. In addition, as mentioned above, bone marrow cells are cycling, and induced deletions either induce cell killing and/or are lost from the daughter cells at mitosis. Thus, any observed deletions must have been produced in the cell cycle immediately prior to the metaphase analyzed. The effect of cumulative exposures is not readily explicable. These results cannot be interpreted as showing that styrene is clastogenic in *vivo.*

Norppa *et al.* (1979) studied chromosome aberration induction in Chinese hamster bone marrow cells following inhalation of styrene oxide. The exposures were 25, 50, 75 or 100 ppm styrene oxide for 2 and 4 days (total exposure 9 h or 21 h, respectively) and for 20 days with 25 ppm. A single i.p. injection with 500 mg/kg was also given. Animals were sampled immediately after the end of the inhalation, or 24 h after injection. No increase in aberrations was observed for any of the inhalation groups, although, as described above, the use of long-term exposures when cycling bone marrow cells are being studied is open to criticism. Following i.p. treatment no aberrations were seen in cells sampled 24 h after treatment, but an increase was reported in bone marrow cells obtained from animals that died 18-22 h after treatment. This could support the contention that the aberration frequency is dependent upon the proportion of 1st division mitoses in the analyzed population. It could also reflect secondary effects due to the fact that the animals were dead when samples were taken.

An addition to this study was the measurement of the levels of hepatic drug metabolizing enzymes following styrene oxide exposure. The results seemed to indicate that Chinese hamsters have a greater epoxide hydratase activity than other rodents. However, this would need a more definitive analysis before firm conclusions can be drawn.

Norppa *et al.* (1980b) also conducted a study on the effects of styrene inhalation exposures on the induction of chromosome aberrations in Chinese hamster bone marrow cells. Exposures were 300 ppm styrene for 4 days or 3 weeks (6 h per day, 5 days per week) and bone marrow samples were taken immediately after the termination of exposure. There was no increase in chromosome aberrations for either treatment, although the assay with cycling bone marrow cells is not acceptable for concluding that the response was negative, as discussed above. In addition, there was no increase in aberrations when styrene exposures were given in combination with 15% ethanol in the drinking water.

A further study by Norppa (1981) was designed to determine if styrene could induce a clastogenic response in mice in contrast to their results in Chinese hamsters because of reported reduced levels of epoxide hydratase in mice. Male mice (C57BL /6) were given a single i.p. injection of styrene (250, 500, 1000 and 1500 mg/kg), and micronuclei analyzed in polychromatic erythrocytes 30 h after treatment. The authors report an increase in micronuclei in the styrene-treated animals. However there is no dose response, the increases are small, and, in part, are shown by the authors to be statistically significant because of a very high number of cells analyzed in control animals compared to treated animals. This study is inconclusive.

Sinha *et al.* (1983) conducted a study of the possible clastogenic effects of long-term exposures in rat bone marrow cells. Exposure was to 600 or 1000 ppm styrene vapor for one year (6 h per day, 5 days per week). Bone marrow cells were sampled at the end of the exposure. There was

no increase in aberrations in the styrene-treated groups compared to the control. However, this study suffers from the misconception that chromosome aberrations can be detected in cycling bone marrow cells following a one year exposure without resorting to chromosome banding techniques. Even then the loss of aberrant cells by cell killing from the analyzed population make this study unacceptable.

Sbrana *et al.* (1983) reported studies designed to relate the effect of styrene biotransformation on the induction of chromosome aberrations in mouse bone marrow cells. CD-1 male mice were treated orally with 500 mg/kg for 4 days or 200 mg/kg for 70 days. Bone marrow samples were taken 24 h after the end of styrene treatment. There was no increase in chromosome aberrations following either treatment, but as explained such an exposure regime is not appropriate when chromosome aberrations are being analyzed in cycling bone marrow cells. In support of their conclusion that styrene is not clastogenic *in vivo,* the authors studied the levels of several styrene metabolites in urine samples, and found that equivalent amounts of all were excreted in 24 h on the 1st or 70th days of treatment with 200 mg/kg styrene. In addition styrene-7,8-oxide (150 mg /kg) treatments were administered orally, and the blood levels followed. A peak blood level of 6 μl/ml was found 20 min. after treatment and then decreased to undetected levels at 1 h. However, when styrene-7,8-oxide levels in the blood were analyzed in the animals given 200 mg/kg for 1 or 70 days, the amounts were undetectable.

(ii) Sister chromatid exchanges

Three papers by Conner *et* a/. (1979,1980a, 1980b) report studies on the induction of SCE by styrene in mouse bone marrow cells, alveolar macrophages, and regenerating liver cells. In the first study BDF_1 male mice were exposed by inhalation of 565 ppm styrene for 4 days (6h/day). BrdU was injected into animals every hour for 9 h on the last day of styrene exposure in order that differential chromosome staining can be obtained. The cells analyzed were bone marrow cells from non-hepatectomized mice and liver and bone marrow cells from hepatectomized mice. There was a significant increase in SCE in all cells (approximately four-fold).

In the second study (Conner 1980b) SCE were analyzed in bone marrow cells, alveolar macrophages and regenerating liver cells exposed to different styrene concentrations for different periods of time. The exposures were by inhalation at 104, 387, 591 and 922 ppm. Exposures were for 4 days (6 h per day) for all concentrations and also for 1 and 2 days for the highest concentration. There was an increase in SCE for all cell types at exposures of 387 ppm and above. The increase was linear with increasing concentration. For the exposure of 922 ppm there was no increase after 1 day of exposure, a small increase for 2 day exposures, and a clear response for the 4 day exposure. The maximum increase was about 3-fold compared to the control. The increase in mean SCE frequency was in several instances due to an increase (but only for a small number of cells) of cells with high frequencies of SCE compared to the contfol. This suggests that some fraction of the treated population is sensitive to SCE induction, and this could affect the implications of the use of the data for extrapolation. These same data are discussed further by Conner *et al.* (1980a), but no new data are presented.

Norppa *et al.* (1979) studied the induction of SCE in Chinese hamster bone marrow cells following styrene oxide inhalation. The concentrations were 25, 50, 75 or 100 pm given for 2, 4 or, for the lowest concentration, 20 days. The BrdU treatment necessary for chromosome differentiation was performed *in vitro* for 28-43 hours. A single i.p. injection of 500 mg/kg was also given, with sampling 7 h after treatment. There was no increase in SCE frequency in the styrene oxide groups (inhalation or i.p. treated) compared to the control. As mentioned above, where a discussion of the chromosome aberration induction was presented, the lack of activity of styrene oxide was suggested to be due to a high activity of epoxide hydratase in Chinese hamsters.

Summary of Rodent *in vivo* Assays

Despite some inadequacy in the protocols used for *in vivo* cytogenetic assays, it appears that styrene does not induce chromosome aberrations in mouse bone marrow cells. Styrene oxide induced a low frequency of aberrations in mouse bone marrow cells, but not in rats or Chinese hamsters, possibly as the result of different metabolizing capacities of different species. Styrene induces increases in SCE in mouse bone marrow cells, alveolar macrophages, and regenerating liver cells. However, S.O. does not induce SCE in Chinese hamster bone marrow cells, again possibly due to a difference in metabolizing enzymes in the two species. The overall results are generally inconclusive, and further studies are clearly indicated.

b. Human lymphocyte assays

General discussion of population monitoring studies

Before discussing the published literature on the frequency of chromosome aberrations in peripheral lymphocytes of persons occupationally exposed to styrene, it is important to discuss some general features of the assay, and factors that can influence the interpretation of the data. A more complete discussion can be found in the review by Preston (1984).

The sensitivity of the lymphocyte assay for detecting possible increases in chromosomal aberrations in persons occupationally exposed to chemical agents will be relatively low. Peripheral lymphocytes are non-cycling cells, residing in G_0 (pre DNA synthesis; equivalent in G_1 in cycling cells). They are initiated to enter the cell cycle by treatment *in vitro* with a mitogen such as phytohemagglutinin. The first *in vitro* cell cycle consists of a long G_1 stage (mean duration approx. 18 h) and an average total cycle time of 48 h. The majority of clastogenic chemical agents induce aberrations in DNA-replicating (S-phase) cells or in cells that pass through the S-phase between treatment and observation at metaphase. The aberrations will be of the chromatid-type. Thus, for peripheral lymphocytes any DNA damage that is induced will not be converted into aberrations until the cells are established in culture, and progress to the S-phase. Only unrepaired DNA damage that remains in cells when they reach the S-phase will be involved in aberration production, and since DNA repair can take place in G_0 lymphocytes and in G, following mitogenic initiation, only some proportion of the induced DNA damage can result in aberration formation. The same argument applies to SCE induction, since SCE result from errors of DNA replication, enhanced with templates containing damaged DNA. The frequency of aberrations and SCE will not be directly related to exposure. In contrast, the analysis of chromosome aberrations in lymphocytes of persons exposed to ionizing radiation can be used as a biological dosimeter, since aberrations are induced in the G_0 peripheral lymphocytes themselves.

In order to determine whether there is an increase in chromosome aberrations or SCE in an occupationally exposed population group, it is necessary to compare the aberration frequency in this group with an unexposed control group. However, there are several factors that are known to influence aberration or SCE frequencies, and these have to be considered when selecting the exposed and control groups for study. Confounding factors include age, smoking, and environmental and medical exposures to potentially clastogenic agents. In addition, there are quite possibly other confounding factors that have not been determined *(e.g.* metabolic differences). For this latter reason, the study groups should be large (greater than 20 persons in each) such that any possible confounding factors will have a small probability of being represented in only one group, thereby biasing the data. Any population monitoring study should include sufficient individuals and cells per individual to provide reasonable power to the statistical analysis (for a discussion see Bloom *et al.* 1981).

Because of the relatively low sensitivity of the assay, an observation of an increase of aberrations or SCE in the exposed group compared to the control indicates an exposure (not the dose level), but if no increase in aberrations or SCE is seen in the exposure group it cannot be concluded that there has been no response. In addition, if the clastogenicity of a single agent in an occupational environment is being assessed, then other potentially clastogenic agents cannot be present in the same workplace. This is generally not the case with industrial styrene exposures.

One additional cautionary note has been discussed above (page 25). It has been shown that "fragile" chromosome sites can be expressed in low folate tissue culture media such as TC199. It is recommended that such media not be used in chromosome aberration assays, particularly population monitoring studies.

Even if these various factors are heeded in a monitoring study, the results obtained still have to be interpreted with caution; this is frequently not the case for published studies.

(i) Chromosome aberrations

The first reported study of the possible cytogenetic effects of styrene exposure to humans is that of Meretoja *et al.* (1977). Ten persons employed in three different plants manufacturing polyester plastic products formed the "styrene-exposed" group. Employment in these plants ranged from a few months to 8.5 years. The control groups consisted of five persons with no known styrene exposure. The level of exposure to styrene was estimated from the mandelic acid level in urine samples, and ranged from 50-3200 mg mandelic acid per g of creatinine. The concentration of mandelic acid varied greatly between individuals and with time of sampling. The lymphocytes were cultured in TC199 that for this small sample is inappropriate (see page 11). This is emphasized by the fact that the aberrations recorded were chromosome-type breaks, not expected to be induced by styrene. It should be noted that the aberration shown in the authors Fig. 1 is a dicentric, but no dicentrics are recorded in the results. The results indicate an increase in "chromosome breaks" in the styrene group. However, there is no relationship of aberration frequency to estimated exposure, and no information on other chemicals in the workplace is given. With these factors, together with the fact that the sample sizes are very small, and the aberration type observed is difficult to explain, this study cannot be considered to be informative with regard to the effects of styrene on aberration induction in lymphocytes.

Fleig and Thiess (1978) studied the induction of chromosome aberrations in workers from different occupational groups that had the potential for being exposed to sty-

rene or polystyrene. The first (5 persons) were involved in styrene manufacturing (average exposure years 21.6) with low mandelic acid concentrations, where assayed. There was no increase in aberrations compared to the frequency in 20 controls. The culture time was 72 h, which is too long to insure a high frequency of 1st division metaphases, and so the results are not unequivocal. The second group (12 persons) worked in a polystyrene manufacturing plant (average exposure year 20.3) with mandelic acid concentrations of 5-100 mg/1 urine. There was no increase in aberrations compared to the control group (that was the small one used for comparison to all occupational groups). Again the 72 h culture means that a cautious interpretation of the data is required. The third group (14 persons) were employed in plants processing unsaturated polyester resins (average exposure years 7.9) with mandelic acid concentrations of 117 to > 1500 mg/1 urine—the highest levels for the different groups. There was a small, but significant increase in aberrations, from 2.1 for controls to 5.3 for the test group. The types of aberrations are not given, and so a complete evaluation is not possible. It is very important to note that there was also expo-- sure to styrene oxide and a range of solvents, and thus it certainly cannot be concluded that styrene exposure induces the reported small increase in aberrations. More work is clearly indicated before any conclusions can be reasonably drawn.

It certainly cannot be concluded that styrene exposure induces the reported small increase in aberrations

The study of Meretoja *et al.* (1978) represents a restudy of the same persons reported in Meretoja *et al.* (1977). The same criticisms apply to the restudy as were presented above for the original study—the small size of the study, the fact that no description is given of other chemicals in the workplace, and TC199 was used. Similar results are reported with an increase presented for total aberrant cells in the test group compared to control. However, it appears that the gaps are included in the category of aberrations, and the control group was not resampled—the aberrant cell frequency was simply the one obtained one year previously. No conclusions on the clastogenicity of styrene can be drawn.

Hogstedt *et al.* (1979) conducted a small study on persons employed in a plant manufacturing fiberglass-reinforced polyester resin boats. The workers were probably exposed not only to styrene but also to a range of other agents that are or have the potential to be clastogenic. The six members of the test group had been employed in the plant for 0.5 to 10 years and mandelic acid concentrations in 9 workers (not described as including those in the test groups) were 225-2100 mg/g creatinine. The frequency of aberrations was measured and compared to a control group of 6 persons. The authors report that there was an increased frequency of aberrations in the test group compared to the control, but there was overlap in frequencies between individuals in the two groups, and the difference in means for "breaks" was very small. If the difference is indeed significant, the increase clearly cannot be attributed specifically to styrene exposure. In fact, no definitive conclusions can be drawn from this study.

Theiss *et al.* (1980) analyzed chromosome aberrations in a group of persons employed in a polyester resin processing pilot plant. The average styrene concentration was 58.1 ppm, and in 4 persons analyzed the mandelic acid concentration was 20-50 mg /lurine. There was no difference in aberration frequency in the test group (24 persons; years of exposure from 4-27) compared to the control group (24 persons). The time of lymphocyte culturing was 72 h that makes the results equivocal. However, it is unlikely that a proportion of 2nd division cells analyzed would alter the authors' conclusion.

Andersson *et al.* (1980) conducted a fairly large study on the frequency of chromosome aberrations in persons working in a plastic-boat factory. In this workplace it is likely that there will be exposure to styrene, as well as to a variety of other unspecified chemi- cals. The exposures to styrene were based on air concentra- tions. A total of 36 persons were included in the "exposed" group; 22 in the low level group, and 14 in the high level group. The low exposure group had mean total styrene exposures (concentration x no. of years) of 137 (range 6- 283); the high exposure group had mean total styrene exposures of 1204 (range 710-1589). The mean employment time for the low group was 3.2 years, and for the high group 7.6 years. The control group consisted of 37 persons, who worked in offices, workshops and the assembly shop. Cells were cultured in Ham's F10 medium (not a low folate medium) for 66-68 h. This culture time, as the authors point out, will mean that a high proportion of cells will be in their 2nd *in vitro* metaphase. There was a small increase in total aberrations in the exposed group compared to the control (7.9% vs 3.2%), and this increase was

largely due to chroma- tid and isochromatid deletions, the types of aberrations most frequently induced by the majority of chemical agents, including alkylating agents. There was no difference in aberration frequency between low and high exposure groups, although the authors claim a linear dose response within the low exposure. Observation of the data does not support this conclusion. The increase in aberrations observed in the exposed group cannot be deduced to be caused by styrene, because their is no dose response with styrene exposure, and styrene is not the only agent in the workplace environment that has the potential to be clastogenic.

Watanabe *et al.* (1981) conducted a fairly small study on the frequency of chromosome aberrations in persons employed in a fiber-reinforced plastic boat factory and in a plant manufacturing polyester-resin boards. The air styrene concentration in group 1 was < 70 ppm (average employment was 4.8 years) and in group 2 was 30-40 ppm (average employment 4.1 years). The mandelic acid concentrations were 90-2640 mg/ g creatinine in group 1 and 300-1360 mg/ g creatinine in group 2. A total of 16 persons were included in the exposed group, and 13 total control persons. A small number of cells were analyzed (a maximum of 50 per person). There was no difference in total aberration frequencies between persons in group 1 and their comparative control group, and similarly for group 2. The information is incompletely presented, and the number of cells is too small for definitive conclusions. However, as it stands there was no effect of styrene or other chemicals present in the workplace on chromosome aberration frequency compared to a control group within the limits of the assay, even though the styrene levels were considerably higher than in studies reporting a positive response.

The study of Dolmierski *et a/.* (1983) need not be considered in any detail, because although they looked at 30 persons employed in the production of laminated styrene plates, only 2 controls were analyzed, with a total of 90 cells. It is not possible to determine the relevance of the aberration frequencies in the exposed group' when there is no suitable control for comparison.

Camurri *et al.* (1983) studied the frequency of chromosome aberrations in the lymphocytes of a group of persons employed in 6 different plants involved in the reinforced-plastics industry. The styrene concentrations measured in the plants ranged from 30 to 400 mg/ mc, and the mandelic acid concentration in the urine ranged from 45 to 1108 mg/ 1. The exposed group consisted of 25 persons and the control group consisted of 22 persons, that were age-matched to the exposure group. The cells were cultured in RPM1 1640 for 50 hours. The frequency of aberrations was reported to be increased in the exposed group compared to the control. The aberrations were classified as chromatid-type and chromosome-type. However, no distinction was made between gaps and deletions, and judging from the frequency in the controls, gaps were included as aberrations. The frequency in controls was about 10%, compared to values of about 2% reported for "true" aberrations in many published studies that were adequately analyzed. In addition, the total % aberrations (? including gaps) was considerably higher than any other values reported for persons employed in the styrene industry. These results are not readily explicable, and cannot be considered reliable. In addition, there are several other chemicals to which persons would be exposed in the plants involved. The authors conclude that styrene is responsible for their reported increases in aberrations—there is no information to support this statement. There is no relationship between "aberration" frequencies and reported styrene exposure.

Hogstedt *et al.* (1983) measured the frequencies of micronuclei in the lymphocytes in persons employed in the manufacture of reinforced polyester resin. The mean styrene concentration was 13 ppm (range 1-36 ppm) and the mean mandelic acid concentration was 65 mg/g creatinine (range 9-316 mg/ g creatinine). The exposed group consisted of 38 men and the control group included 20 men—both groups contained individuals who had recent radiographic examinations and routinely took drugs for medical reasons. These factors usually negate inclusion in population monitoring studies. A micronucleus was classified as being stained similarly to the main nucleus, but less than one-third of the size of the main nucleus. This is a very unreliable classification, since micronuclei represent the acentric fragments associated with chromosome aberrations, or occasionally whole chromosomes; the likelihood of their being of a size equal to one-third of the main nucleus is exceedingly small or probably zero. The results as presented for each individual tested indicate that if there is an increase in micronuclei in the exposed group it is very marginal—there is considerable overlap between the "micronucleus" frequencies in exposed and control groups. There is no relationship to exposure. There are other chemicals present in the workplace and no account is taken of these. These results do not add reliable information on the possible clastogenic effect of styrene.

Watanabe *et al.* (1983) conducted a study on chromosome aberrations in lymphocytes of persons exposed to styrene in a plant utilizing fiber reinforced plastics. They state that there was no exposure to other chemical agents

but no evidence was provided for this statement. The styrene exposure was 40-50 ppm and the mandelic acid levels ranged from 0-1041 μg/ ml urine (mean 332 p,g/1). There were 18 persons in the exposed group from two different workshops, but only 6 persons in the control group, clearly a non-matched control. The cultures were grown in RPMI 1640 for 50 h; BrdU was added 24 h after mitogenic initiation so that 1st division could be analyzed following the fluorescence plus Giemsa staining technique. No increase in aberrations was observed in the exposed group compared to the control, either when total persons sampled were included or when smokers and non-smokers were separated. This study was not definitive because of the limited control group, but otherwise it was well-conducted.

Nordenson and Beckman (1984) studied chromosome aberrations and micronuclei in peripheral lymphocytes of persons employed in a factory where polyester reinforced with glass fiber was used. The exposed group consisted of 15 men and the control group contained 13 men. The mean air styrene concentration was 24 ppm with a mean mandelic acid concentration of about 1 mmol/ 1 urine. The lymphocytes were cultured in RPMI 1640 for 64-68 h; a time of sampling when a high proportion of 2nd division metaphases would be expected. The frequency of chromatid and chromosome breaks was not increased in the exposed group compared to the control. This result was the same if smokers and non-smokers were compared in the two groups. The frequency of micronuclei was reported to be increased in the exposed group, which is a surprising result since aberration frequencies were not increased. A single sampling time (96 h) was used for the micronucleus assay, and this could influence the results if there is a difference in frequency with cell division rate, as has been reported—the more divisions a cell population goes through the lower the micronucleus frequency. The influence of other chemicals in the workplace environment was not discussed. These data do not allow for an assessment of the clastogenic effects of styrene exposure, although the aberration data are more conclusive than the micronucleus data.

The study of vanSittert and deJong (1985) cannot be used to study the clastogenic effects of styrene because workers were also exposed to propylene oxide and benzene. It is interesting to note that the frequencies of chromosome aberrations were not increased in the exposed group compared to the control. The styrene exposures were between 0.1 and 1.4 ppm over a four year period. The aberration frequencies were also measured in the same individuals pre-employment—the best control group. The methods used are not sufficiently well-described to allow review.

(ii) Sister chromatid exchanges

In five of the monitoring studies described above sister chromatid exchanges were also analyzed. Since there are different criteria and methods that need to be applied to SCE analysis they are considered separately from the aberration assays. Details of the study group and exposure levels can be found in the section above on chromosome aberrations.

Meretoja *et al.* (1978) analyzed SCE in 11 persons employed in the reinforced plastics industry, and 3 control persons. Cells were cultured for 66-68 h and 2nd division metaphases analyzed. There was no difference in SCE frequency between exposed and control groups. However, the study was small, and other chemicals were present in the environment. The lack of an increase in SCE is in contrast to the authors reported increase in aberrations. The aberration data, however, are inconclusive as discussed above.

Andersson *et al.* (1980) reported a slight increase in SCE in the exposed group compared to the control group. However, since other chemicals were present in the workplace, the difference was not significant when the comparison group was persons employed in a workshop, and the frequency was lower in exposed smokers vs. non-smokers, it is reasonable to conclude that styrene exposure did not increase the SCE frequency.

Similarly, Watanabe *et al.* (1981) did not find an increase in SCE frequency in the exposed group (fiber-reinforced plastic factory) compared to the control. This was a rather small study with only a partially matched control group (age and sex).

Camurri *et al.* (1983) also studied SCE frequencies in the groups in which they measured chromosome aberrations. They reported an increase in SCE for persons employed in workplaces with styrene concentrations over 200 mg/mc. There was not a significant increase in SCE frequency in persons employed in those workshops with lower styrene concentrations. The SCE frequency was higher than normally reported for control groups (-11.70 per cell) when BrdU at 1 .tg/ml is used to obtain differential chromosome staining. There was no clear relationship between styrene air exposures and SCE frequency. The most important feature when attempting to interpret this study is that other agents that are clastogenic (or have the potential to be) are present in the workplace, and so styrene cannot be specifically indicated as the agent that causes an increase in SCE.

A second study of Watanabe *et al.* (1983) also analyzed SCE and aberration frequencies in a group of persons (18) employed in fiber-reinforced plastic boat factories compared to a control group (6 persons). The SCE frequency was not increased in the total exposed group compared to the control. The authors reported that there was a significant increase in exposed smokers compared to control smokers (9.6 vs. 8.4). However, the control sample consisted of only three persons, and other agents were present in the workplace. It is also interesting to note that the lowest frequency of SCE was for the exposed non-smokers. It appears that the increase in SCE noted by the authors for exposed smokers was a feature of the small number of samples (and cells analyzed), the lack of matching or comparisons for factors other than smoking, and representative of the increase that smoking normally produces in SCE frequencies in lymphocytes of smokers (about 1 SCE per cell).

ADDITIONAL NOTE

In a recent publication, Maki-Paakkanen (1987) measured the frequencies of chromosome aberrations, micronuclei and sister chromatid exchanges in lymphocytes of persons exposed to low levels of styrene. There were 21 individuals in both the test and control groups, with 15 smokers and 6 nonsmokers in each group, and with similar mean ages. The workers had been employed in a plant manufacturing reinforced plastic products for 1 to 25 years. The mean styrene concentration was 98 "x" mg /m3 air (range 34 to 263 mg/ m"x "), and the urinary mandelic acid levels varied from 0 to 7mM mandelic acid per litre of urine. Lymphocyte cultures were established in RPMI 1640, and incubated for 50 h for aberration analysis, 68 h for SCE analysis and 82 h for the measurement of micronuclei. These are all acceptable sampling times. There were no differences in the frequencies of chromosome aberrations, SCE or micronuclei between the two groups. There was an increase in SCE for smokers, but this was similar for both exposed control groups. There is no identification of other chemicals in the workplace. The sample sizes in this study are reasonable, and it is generally well-conducted—the authors conclusions appear to be valid.

SUMMARY OF POPULATION MONITORING STUDIES

The fairly large number of cytogenetic monitoring studies on persons employed in various workplaces where styrene exposure is likely do not provide data that can be used to specifically determine whether or not styrene exposure induces chromosome aberrations or sister chromatid exchanges in peripheral lymphocytes. For chromosome aberrations some authors report a positive response and

others a negative response. However, a variety of factors make these studies inconclusive. The protocols themselves are generally not appropriate and because of number of samples, time of sampling, tissue culture medium, classification of aberrations, and failure to account for possibly confounding factors in comparing exposed and control groups, the studies could result in false positive conclusions or false negative conclusions. The fact that other agents that are or potentially are clastogens are present in the workplace does not allow for unequivocal conclusions about the effects of styrene.

The SCE studies are generally negative, *i.e.* there was no difference in frequency between exposed and control groups. It is easier to conduct a satisfactory protocol for SCE analysis because 2nd mitoses are analyzed by necessity, the analysis is simpler than for chromosome aberrations, and only one type of end-point is observed. However, several other features can affect the interpretation. Although the studies reported are negative, several factors make the interpretation equivocal: confounding factors not generally adequately considered, sample sizes small, and other agents in the workplace environment not considered. Therefore, no firm conclusions can be drawn. Additional studies, more definitively planned, need to be conducted.

OVERALL SUMMARY OF STUDIES ON THE CYTOGENETIC EFFECTS OF STYRENE

This review has taken the approach of evaluating each reported study of the potential cytogenetic effects of styrene separately. This was deemed necessary because there are so many specific features in conducting and interpreting such assays. This makes for some repetitiveness, but this does not serve to highlight some particular issues.

The assays cover rodent cells and human lymphocytes treated *in vitro* and rodents and humans exposed *in vivo,* with chromosome aberrations and sister chromatid exchanges as end-points. The *in vitro* assays in some cases have employed exogenous metabolic activation systems (generally S-9 from livers), and in others (particularly lymphocytes) have assessed *in vitro* effects in the absence of activation.

The magnitude of any cytogenetic effects of styrene in *in vitro* assays is difficult to ascertain because of the particular protocols and/or tissue culture media used. However from a consideration of all the available data from *in vitro*

assays, it can be concluded that the effectiveness of styrene at inducing chromosome aberrations lies somewhere between marginal and non-effective in rodent cells and cultured human lymphocytes. Styrene does induce sister chromatid exchanges in cultured human lymphocytes, but not in Chinese hamster cells *in vitro,* even in the presence of metabolic activation. This might be the result of different metabolic activation/deactivation capabilities of different species.

Styrene oxide, a primary metabolite of styrene, and other styrene metabolites are considerably more effective than styrene at inducing chromosome aberrations and SCE in *in vitro* cell systems. This is an interesting observation, but does not allow for speculation of the effects of styrene *in vivo* since it is not clear how effectively styrene is metabolized, and what proportion of the metabolites, or styrene itself, actually reach the cells that are analyzed, namely peripheral lymphocytes. In addition, the balance between activation and deactivation will affect the amount of the different metabolites, and will be highly variable among individuals. This point is exemplified by *in vivo* studies with mice and Chinese hamsters. Styrene appears to induce SCE in mouse bone marrow cells, but is ineffective at inducing either aberrations or SCE in Chinese hamster bone marrow cells. It could well be that an increased level of epoxide hydratase in Chinese hamsters causes a more rapid metabolism of styrene oxide, thereby reducing the effectiveness of styrene through the 7,8-oxide. For humans the kinetics of metabolism have not been studied directly, and so whether a response would be expected to be similar to Chinese hamsters or mice cannot be accurately determined. However, Ramsey and Anderson (1984) provide some data that suggest that inhalation pharmacokinetic models for rats could be used to extrapolate to man.

The studies involving the induction of chromosome aberrations and SCE in human peripheral lymphocytes following exposure in the workplace to styrene (as well as to a variety of other chemicals) are generally considered to be the most relevant for determining the potential risk of styrene exposure. Unfortunately, the published data, as reviewed above, fall rather far short of allowing any risk estimation. Not only are the studies often fraught with technical problems (inadequate protocol, small sample size, only superficial control matching, and uncontrolled sources of variation) but the exposure information on chemicals other than styrene is completely lacking. In addition, the potential cytogenetic effects of these other agents is not determined even though *in vitro* and *in vivo* data are sometimes available. The studies reported cannot be used to show that styrene alone can induce chromosome aberrations in human peripheral lymphocytes *in vivo,* and although the SCE assays have some of the same problems, the results are negative, *i.e.* no increase in SCE for "styrene exposed" groups compared to controls.

The question of the genotoxicity and carcinogenicity of styrene exposures to humans is a very important one, and certainly merits careful study. It is essential to conduct adequate and informative studies, particularly to sort out many of the confusions that have arisen as a result of taking the conclusions presented in the published literature at face value.

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47 1. Note on the use of TC 199

48 An important note should be made here about the use of TC 199, a low folate tissue culture medium. There is a reasonable probability of an influence of this medium on the results of chromosome aberration assays with lymphocytes, not so much where a single donor is used and treated groups are compared to the control, from the same donor, but for studies with multiple donors, particularly in populations in monitoring studies [discussed in Section 2b(i)]. TC 199 was the medium in which fragile-x syndrome was originally observed by Sutherland (1979), and subsequent studies by Reidy *et* al. (1983) and Li et al (1986) have shown that the background frequency of aberrations is higher when lymphocytes are grown in low folate medium. There is also considerable variation in individual responses, perhaps reflecting variations in cellular or serum folate levels. Thus it is considered to be an incorrect practice to use TC 199 or similar low folate media for aberration studies.

A Critical Review of the Reproductive and Developmental Data on Styrene

Nigel A. Brown, Ph.D.
Teratology Section Head, Medical Research Council
St. George's Hospital Medical School, University of London (UK)

The potential developmental toxicity of styrene has been tested in several mammalian experimental species, but only one study is of good quality. Throughout all studies, there is no evidence for teratogenicity. There are reports of increases in embryonic, fetal, and neonatal death, and perhaps skeletal variations, in the offspring of styrene-exposed animals but the evidence is not convincing, except at doses which are maternally toxic. Similarly, styrene oxide has induced embryo-fetal death and retardation, but no malformation, at doses profoundly toxic to the dams. There is clearly a lack of well replicated studies, but the bulk of information suggests that styrene does not exert any specific developmental toxicities.

Styrene has been reported to induce malformations in the chick embryo. This does not necessarily suggest a specific developmental toxicity and should not be extrapolated to mammals. Effective doses were significantly embryolethal.

Initial human studies suggested associations of styrene exposure with congenital central nervous system (CNS) malformation and spontaneous abortion. These have been disproved by subsequent more extensive investigations by the same investigators. There was no evidence in these studies that styrene exposure was associated with any congenital malformation, nor abortion. One study has reported a 4% decrease in the birth weight of children born to women exposed to high levels of styrene and other solvents during pregnancy. The number of women was small and the reduction was not statistically significant.

Though styrene has sometimes been referred to as teratogenic (causes malformations) or as a potential human developmental toxicant, the only previous comprehensive review of the data, published in 1981, did not reveal any definite specific effects. Dr. Brown re-reviews the data up to the present, including the unusually large number of Soviet studies. He finds no evidence for teratogenicity in any of the studies and little indication that styrene can exert any specific developmental or reproductive toxicity. He notes that while initial human studies linked styrene exposure with congenital malformation and spontaneous abortion, these findings were later disproved by subsequent more extensive investigations by the same researchers.

There are no reports of styrene adversely affecting the fertility of female experimental animals, but only one (low-dose) study was designed specifically for this purpose. Some data suggest styrene exposure may affect the estrous cycle in rats and the menstrual cycle in women. The experimental data are inadequate, and there are conflicting human studies. Ovarian pathology in rats has been reported, but not confirmed.

There are no reports of styrene adversely affecting the fertility of male experimental animals, but again, only one specific (low dose) study has been performed. A recent small study suggests a reduction in sperm counts and testicular pathology in rats treated with a high oral does of styrene, but this may be complicated by hepatic toxicity. Several other studies have not observed testicular pathology. A recent examination of sperm parameters of men exposed to styrene suggested an increase in abnormal forms, but the data were weak and no firm conclusion can be drawn.

High level inhalation exposure of rabbits induces some changes in brain dopamine which may affect the function of the hypothalamus and pituitary. Abnormalities of pituitary secretion in women exposed to styrene have also been suggested. These effects may be connected with the putative effects on the menstrual cycle.

Overall, there is little indication that styrene can exert any specific developmental or reproductive toxicity. Any putative effects on female reproduction

could have a CNS site of action, which is compatible with the known neurotoxic actions of styrene.

Various aspects of styrene toxicity have been well studied and there are several reviews [1,2,33]. The potential reproductive and developmental toxicity of styrene has received relatively little attention. The aim of this review is to collect together all the relevant published data, evaluate its significance and identify gaps in our knowledge. There are two areas, which might be considered part of reproductive toxicity in the broadest sense, that I will not review: germ cell mutation and trans-placental carcinogenesis. These are more properly dealt with as aspects of genotoxicity. I would only say that although chemically-induced germ cell mutation can clearly give rise to developmental toxicity [5], there is no relevant information for styrene.

Published opinions on the reproductive and developmental toxicity of styrene are contradictory. The only previous comprehensive review of data, published in 1981[6], did not reveal any definite specific effects. However, it is common to see styrene referred to as "teratogenic", and a recent survey[7] classifies styrene as a "potential human developmental toxicant", with death and malformation as the reported developmental toxicities.

A brief review of human exposure levels, and acute toxic concentrations, will help to put the studies reviewed here into context. By far the most common route of human exposure is by inhalation. The American Conference of Governmental Industrial Hygienists recommended, in 1987, a threshold limit value-time- weighted average of 50 ppm, and a short-term exposure limit of 100 ppm[1]. The equivalent recommendations from the US Occupational Safety and Health Administration, set in 1989, are 50 ppm and 100 ppm. It has been stated that the lowest reported acutely toxic concentration in humans (nasal and eye irritation) is between 400 to 600 ppm[1].

Styrene has a low acute toxicity in experimental animals. The oral LD50 in rats is about 5000 $mg.kg^{-1}$ and about 320 $mg.kg^{-1}$ for mice[1]. By inhalation, the lowest LD50 reported for rats is 5000 ppm, for 8 hours. With chronic inhalation by rats, reversible changes in nasal mucosa have been observed inconsistently at 50 ppm, and clearly at 150 ppm.

I quote concentrations of styrene in air in parts per million (ppm) throughout this report. Where original sources used $mg.m^{-3}$ these have been converted using the factor 1 ppm = 4.3 $mg.m^{-3}$. In all cases, concentrations are given in the original units on their first mention in the report. When exposures are quoted as ppm, the route of administration was by inhalation, unless otherwise stated.

The following sources were used in literature searching; Chemical Abstracts (both printed and on-line); Medline (CD version); ETIC (ON-LINE); and REPROTOX (on-line). These searches covered the period 1960 to the end of 1989. For January to April 1990, "Current Contents" was used.

There are a large number of Russian language publications amongst the relevant reports. For all those cited, I examined the original publication. Some are also available as English translations [8; 9;10; 11.]

In general, evaluation of the significance of Russian reports is very difficult. By Western standards, the experimental detail provided is often inadequate, particularly in older reports. This does not necessarily imply that methods used were deficient. The accepted style of the Russian science literature is simply different from that in the West. However, it does mean that many of the findings can not carry real weight because they are unsubstantiated by experimental detail. This is true of many of the reports reviewed here, as discussed in detail for individual studies.

In addition, there may be inconsistencies in the way styrene concentrations are measured, calculated, or reported in some Russian studies. Most Western countries have threshold limit values for styrene of 25 to 100 ppm (about 200-400 $mg.m^{-3}$). The equivalent figure (MAC) in the USSR is 5 $mg.m^{-3}$ (1.2 ppm). Some Russian studies (*e.g.* 12) report symptoms of acute styrene toxicity in rodent studies at much the same levels as reported in Western publications, *i.e.* around 1000 ppm. In sharp contrast, other reports (*e.g.* 10) suggest toxicity at around 10 $mg.m^{-3}$(2.5 ppm). There is no obvious explanation for these perplexing discrepancies. One possibility is the presence of contaminants in styrene, the source and purity of which is not stated in these studies.

Overall, then, it is prudent to be cautious in the interpretation of the Russian studies. Although they are easy to criticize from modern Western standards, it would not be wise to dismiss all as irrelevant, and I have tried to deal even-handedly with these difficulties.

The remainder of the report is divided into five major topic sections. There are generally three sub-sections within each: 1) data from animal experiments; 2) observations on human exposure and 3) an overall evaluation of the available data and gaps in our knowledge.

DEVELOPMENTAL TOXICITY

2.1 ANIMAL

2.1.1 Prenatal death, malformation and retardation

The earliest experimental study of the developmental toxicity of styrene was performed by Ragule in 1974 at the Institute of Industrial Hygiene and Occupational Health, Moscow, USSR [97]. The published report is lacking in experimental detail and is difficult to interpret. Two inhalation studies were performed, using albino rats (strain not stated). The period and frequency of exposure is not clearly stated, but was probably 4 hours/day. It is not stated if controls were sham-exposed. It is reported that several indices of maternal health were measured, but there is no mention of the results.

These studies suggest that there is little effect of styrene on development at maternally toxic doses.

In the first study, exposures were 50, 5 or 0 $mg.m^{-3}$ (11.6, 1.2 or 0 ppm) styrene, throughout gestation. Each test group was 23 animals, with 15 controls. Some were allowed to litter, but numbers are not given. The author reports a significant increase in pre-implantation death and total embryomortality at 11.6 ppm, but no effect at 1.2 ppm. The report makes no mention of counting corpora lutea, so "pre-implantation death" is probably based on number of implantation sites, which is not accepted practice. Since "total embryomortality" seems to include "pre-implantation death" this parameter may also be misleading. The incidence of still-born per female is given as 0.2 ± 0.6 for the two exposed groups (pooled?) compared to 0 for controls, which is reported to be a significant difference, but without statistical analysis. It is not clear if these data are from sacrificed dams or from those allowed to litter. The number of pups dying during the second post-natal week was 1.7, 0.6 and 0 per female in the three groups. There was no increase in malformations in this study.

The second study used 20 animals in each of 3 groups exposed to 5, 1.5 and 0 $mg.m^{-3}$(1.2, 0.35 or 0 ppm). Half were exposed throughout gestation (as above), half during the "first trimester" only. Again, it is reported that there was a significant increase in "pre-implantation" deaths for both exposed groups, with both exposure periods, but no data are given. Post-implantation deaths are also reported to be significantly higher after both exposure levels, throughout gestation only. The control incidence is stated to have been 0, but exposed values are not stated. Embryonic size and weight is reported to be reduced after 1.2 ppm throughout gestation, but again no data are presented. There is no mention of malformations.

The numbers of resorptions per female with total gestational exposure are given as 1.3 and 0.2 for exposed and 0 for control groups, and 0.7 for the higher dose and 0 for controls with the shorter exposure. Although a treatment effect is claimed, it is highly likely that these are within normal variation. The resorption rate is not given for the first study, but control rates of total embryomortality was 15%, pre-implantation death 4% and stillbirth 0%, so re-sorption must have been about 11%; roughly 1 per female (litter sizes are not given). Similarly, in this second study it is reported that total embryomortality was significantly increased to 12.8% by exposure to 1.2 ppm throughout gestation. There was no significant equivalent effect in the first study, where the control rate was 15%.

Few definite conclusions can be drawn from this report. The evidence for any of the claims made is not convincing, and there are inconsistencies between the two studies. No information is given on the maternal effect of exposure, although the conclusions state that 11.6 ppm was accompanied by a "general toxic effect". There is no suggestion that styrene increased the incidence of malformation.

In a study which is reported only in abstract form[20] Efremenko and Malakhovskii, of the USSR, examined the effects of a 9-month exposure to a drinking water extract of polystyrene foam on the reproductive function of rats. The extract contained 0.08 $mg.1^{-1}$ styrene, but other components are not described. It is stated that: the frequency of pregnancy was reduced by 9%; the number of pups born diminished from 9.1 to 6.6/litter; pup weight decreased from 6.1 to 5.6 g; and the exploratory reflex of the pups was inhibited and the defensive reflex was retarded. It is not possible to evaluate the study, as no further details are given.

In 1978 Murray and colleagues of Dow Chemical U.S.A. published a report of an orthodox "Segment 2" study of styrene developmental toxicity in the rat (Sprague-Dawley) and rabbit (New Zealand), with inhalation and gavage exposures [73]. The study was sponsored by the Chemical Manufacturers Association. This is generally a clear report of conventional studies. Rats and rabbits were exposed to

600 and 300 ppm styrene for 7 hours/day by inhalation, and also rats to 150 or 90 $mg.kg^{-1}$ twice daily by gavage.

The exposure of rats to styrene at both dose levels by both routes was accompanied by a significantly smaller maternal body weight gain over days 6-9 compared to sham-exposed controls. This is stated to have been associated with a reduced food consumption, and increased water consumption with inhalation, although data are not presented. There was no effect of exposure on the numbers of implantations and live fetuses or fetal body weight. There was a statistically significant effect on crown-rump length at 300 ppm, but not at 600 ppm, nor with gavage treatment. The incidences of external, visceral and skeletal malformations were not significantly affected by treatment, although these incidences (rather than the numbers of fetuses) are not reported. The report states that the incidence of skeletal variants (mostly lack of ossification) was significantly higher in styrene litters than controls, although within the range of historical controls. None of the data concerning skeletal variations were reported.

The exposure of rabbits to 600 and 300 ppm styrene had no effect on any maternal or fetal parameter, except there was a significant increase in the incidence of minor skeletal variants at 600 ppm, again ossification defects. This incidence is reported to have been within the range of control values "from other recent studies", although, again, no data are presented.

These studies suggest that there is little effect of styrene on development at maternally toxic exposures in the rat, and at 600 ppm in the rabbit. It is difficult to assess the authors' assertion that the significantly higher incidences of minor skeletal variations, observed in both rats and rabbits, are not treatment-related, but within the normal control range, because the data are not reported. There is no consensus in the field on the significance of such skeletal variations, nor on the use of historical control data. The other problem with this study is that the highest exposure to the rabbit was without any maternal effect, which is contrary to current guidelines. It is obviously not possible to conclude anything about potential effects at higher doses, particularly in the light of the "probe" study in which bred rabbits were exposed to 1000 ppm styrene for 7 hours / day for 10 days without any alteration in demeanour or maternal weight gain.

Vergieva and colleagues[122], from the Institute of Hygiene and Occupational Health, Sofia, Bulgaria reported in 1979 on effects of styrene inhalation in pregnant albino rats (strain not given). Rats are reported to have been exposed for 4 hours/day, 5 days/week to 700 $mg.m^{-3}$ (163 ppm) on pregnancy days 2-16 or 200 mg.m3 (47 ppm) on days 2-21. It is not clear how a 5-day exposure cycle is compatible with days 2-16 or days 2-21, but this is not explained. Some of the dams exposed to 47 ppm were sacrificed and examined on day 21, the rest, and all 163 ppm animals, were allowed to litter and the development of the offspring was evaluated. In all cases the number of animals per group was small, from 7 to 12.

On examination at day 21, there was no effect of 47 ppm styrene on pre- or post-implantation death, nor on malformations (of which there were none in exposed or control fetuses). Fetal weight and maternal effects were not reported.

In those dams allowed to litter, pup weights were reduced at 1 and 7 days postnatal in the 47 ppm group (but not 163 ppm), but were stated to be within usual control limits. Numbers of pups in this and subsequent tests are not reported. There was no difference between groups in the behaviour of offspring at 45 and 75 days, as measured by open-field activity or by a noise stress test. There are no details of methodology or results, however. One month after the end of exposure the duration of hexobarbital sleeping time, an index of hepatic xenobiotic metabolising function, was unaffected in both dams and offspring. The only significant styrene-induced effect was an increase in the numbers of erythrocytes, at one month in both dams and progeny. This parameter was measured only in the 47 ppm group. In the 163 ppm offspring, but not the 47 ppm group, haemoglobin was significantly greater than controls.

There are some deficiencies in the reporting of these studies, but their major limitation is the small numbers of animals used. The power of the studies to detect minor changes is very limited. It is reasonable to conclude that there was no major effect of styrene exposure at 163 or 47 ppm on embryomortality, malformation and some aspects of post-natal development. The significance of the haematological changes are difficult to evaluate, but appear to occur in both dams and offspring.

The effect of styrene inhalation on the development of mice and hamsters was reported by Kankaanpaä and colleagues from the Institute of Occupational Health, Helsinki, Finland [51]. Mice (BMR/T6T6) were exposed to 250 ppm 6 hours/day, days 6-16 and sacrificed on day 16 (which is unusual, 18 being conventional). There were 15 control and 13 test animals. Fetal viscera were not examined. The data are not presented in the accepted "per litter" format, so are difficult to evaluate. It is stated that there was a significant increase in the number of dead and resorbing fetuses with styrene exposure; 28 of 104 total implants (incorrectly listed as "number of fetuses"), compared to 21 /115 in controls, $p < 0.10$, Fisher's exact test. This is not a valid conclu-

sion; the level of significance is too low, and the statistical test is inappropriate. Analysis of such data should be on a litter basis, using a non-parametric test.

It is also stated that more malformed fetuses were found in the treated group—2.9% compared to 0.9% in controls. Unconventionally, these percentages must be expressed per implantation, rather than per live fetus, because there were only 94 live fetuses in the control group. There is no statistical analysis, but the percentages translate to 3 malformed of 76 live exposed fetuses, and 1 of 94 controls. Appropriate analysis can not be done without information on litter distribution, but this is very unlikely to be a statistically significant difference. Although maternal weights were taken on days 6 and 16, these data are not reported and there are no other comments on maternal toxicity.

In pilot studies, mice were exposed to 500 and 750 ppm. This resulted in 33% (2/ 6) and 60% (3 /5) maternal deaths, respectively. The fetal death rate in surviving dams is reported as 47% and 95% respectively. With such profound maternal toxicity and small numbers of animals no conclusions should be drawn from these observations.

Chinese hamsters were exposed to 300, 500, 750 or 1000 ppm styrene, 6 hours/day, days 6-18 (confusingly, the authors call the first day of pregnancy "day 1" for hamsters and "day 0" for mice). The number of animals was very low (2, 3, 5 and 7 respectively, with 15 controls). There were no differences between control and 300-750 ppm groups in any reported parameter, and no malformed fetuses in the whole study. The number of dead and resorbing fetuses was 33 of 50 implantations (again, reported as "number of fetuses") at 1000 ppm, compared to 28 of 107 in controls. No objective measures of maternal toxicity are reported to have been made, although the authors state "maternal toxicity was small".

Few conclusions can be made from these studies. Styrene exposure had little effect on development, except at maternally lethal concentrations (500-750 ppm) in the mouse. The increase in embryo-fetal death at 1000 ppm in the hamster can not be interpreted because maternal toxicity data are not provided.

Zaidi and co-workers from the Industrial Toxicology Research Center, Lucknow, India [130] performed a study mainly concerned with the effect of gestational and lactational exposure to styrene on some aspects of brain function (see section 5.1.1). Albino rats (strain not stated) were given 200 $mg.kg^{-1}$ styrene by gavage daily throughout pregnancy. There were no significant effects on the following parameters: number of pups/litter; pup weight at birth and during the first three weeks; protein content of the striatal region; striatal dopamine receptors. A combination of gestational and lactational exposure had some effects, but this was similar to exposure during lactation alone. Conclusions are limited by the small numbers of animals used (3 litters/group).

In another study sponsored by the Chemical Manufacturers Association, Bellies and colleagues report on a three-generation reproduction study of styrene in the drinking water of rats [8]. The concentrations used were 0, 125 and 250 ppm. The objective was to exaggerate possible human ingestion of styrene leached from polystyrene packaging. The higher concentration approaches the aqueous solubility limit of styrene (about 300 ppm), but resulted in relatively low total intakes of styrene; about 14 $mg.kg^{-1}$ for male and 21 $mg.kg^{-1}$ for female rats. These doses had little effect on adult animals. Water consumption was dose-dependently reduced, and after 2 years the body weights of females on the higher dose were slightly, but significantly, reduced.

There were no treatment-related actions of styrene on rat reproduction. No malformations were observed.

The authors concluded that there were no treatment-related actions of styrene on rat reproduction. No malformations were observed in the study. There were statistically significant differences between controls and the high-dose animals in each of the three generations: the F_1 pups had a reduced survival index at 21 days. This was significant when analyzed on an individual pup basis, but not by litter analysis, because all deaths were from 2 of 15 litters. F_2 pups had a reduced survival index at birth, 1,7 and 14 days, but not 21 days. The authors did not offer an explanation. F_2 female fertility was reduced, but the authors argued that this may have been due to the sterility of one male. There was no evidence for this proposition, nor did the authors consider if this might be a treatment effect. F_3 pup weights were lower on days 7 and 14, but not days 1 and 21. For each generation, pup organ weights and ratios, histopathology and cytogenetics were reported not to show "any consistent indication of dose-related differences", although the data were not presented.

Overall, this appears to have been a well designed and conducted study. There was no evidence of a teratogenic

or embryo/fetal lethal effect of styrene at 250 ppm in the drinking water. This exposure, over a lifetime, had a slight effect on female body weight. There was no effect on any reproductive parameter that was consistent across generations. There was some effect on post-natal survival and/or growth of pups in each generation. The effects were small and on different parameters in each generation. Their significance is unclear. An apparent effect on F_2 female fertility may have been a chance occurrence of infertility in a single male, but this cannot be proven.

Styrene oxide

There is only one available study of the potential reproductive and developmental toxicity of styrene oxide. NIOSH-sponsored investigations of the effects of pre-mating and gestational inhalation in the rat, and gestational inhalation in the rabbit, have been published in preliminary[29; 30] and final [112] forms.

Female Wistar rats were exposed to 300, 100 or 0 ppm styrene oxide (106, 106 and 159 rats respectively). 300 ppm was rapidly lethal and exposure was terminated. 100 and 0 ppm were continued for 3 weeks for 7 hours/day, 5 days/week then females were mated, without exposure, to untreated males. Half the dams from each group were then exposed to 100 ppm from days 0-18, 7 hours/day. This gave 4 groups: 0-0; 0-100; 100-0; 100-100.

The exposures were lethal to 16% of animals pre-mating and to 14% during gestation. There were significant reductions in body weights and food consumption in both periods of exposure. There were also treatment-induced lung lesions and changes in organ weights.

The three week exposure had no effect on female fertility, other than a reduction in the numbers of corpora lutea, which was not statistically significant. The pre-mating exposure had no effect on the number of rats pregnant on day 21, but this was severely reduced by gestational exposure: 0-0,85%; 100-0,88%; 0-100,31%; 100-100, 18% (not statistically different from 0-100). This indicates pre-implantation loss of all conceptuses in affected animals. There were no other effects on reproductive performance.

Gestational exposure was associated with reduced fetal size, which reached statistical significance for the 0-100, but not the 100-100, group. There was no increase in malformations in any exposed group, but there were statistically significant increases in ossification defects with gestational exposure.

New Zealand White rabbits were exposed to 50, 15 or 0 ppm (23 or 24 animals per group) from gestation day 1-24, 7 hours/day. The incidences of death during exposure were 79%, 17% and 4%, respectively (the cause of death of the one control rabbit could not be identified). Styrene oxide inhalation also reduced body weight gain, and food consumption (to 50% of control in the 50 ppm group), and caused pulmonary inflammation.

There was a dose-dependant increase in the number of litters with resorptions, although the number of post-implantation deaths per litter was not significantly increased. Fetal weight and length were dose-dependently smaller, but differences were not statistically significant. There was no effect of exposure on malformations, ossification or variations.

These were well-performed and reported studies. The authors concluded that styrene oxide caused reproductive and developmental toxicities at the levels studied, but that it was not established whether these were direct effects, or the result of maternal toxicity. Because non-maternally toxic exposures were not examined it is not possible to state conclusively that styrene oxide only induces reproductive and developmental toxicities with maternally toxic exposures. However, no malformations were observed and most indices of reproductive and developmental function were not affected, even at maternally lethal exposures.

2.1.2 Postnatal behaviour and function

There is very little information on the post-natal sequelae of prenatal exposure to styrene, as illustrated in a recent review which attempts to make a case for post-natal CNS effects but does not present any evidence [119].

As discussed above, Vergieva *et al.* [122] did not observe any effect of inhalation of 47 or 163 ppm on the growth and behaviour of rat offspring, but the study was very small.

The study of Zaidi *et al.* [130] has also been mentioned. Following daily administration of 200 $mg.kg^{-1}$ styrene to rats throughout pregnancy there were no significant effects on the number of pups/litter; pup weight at birth and during the first three weeks; protein content and dopamine receptor levels of the striatal region at 2 and 3 weeks.

There were no reported post-natal effects of 250 ppm styrene in the drinking water of rats, except small, inconsistent effects on survival and growth [8], see above.

In a study of styrene carcinogenicity, mice and rats were administered a high oral dose of styrene on the 17th day of gestation [94]. 0_{20} mice and BDIV rats treated with 1,350 mg.kg' styrene gave birth to offspring which had a higher incidence of pre-weaning mortality than controls. The incidences are reported as 43% *vs.* 22% for mice and 10% *vs.* 2.5% for rats, but actual numbers are not reported and can not be reconstructed from the data provided. It is not possible to distinguish between an effect on the lactat-

ing mothers or one on the offspring. The offspring were studied throughout life but were subject to weekly styrene exposure, so it is not possible to evaluate the effect of prenatal treatment alone. Treatment of C57BL mice with 300 $mg.kg^{-1}$ styrene had no effect on pre-weaning mortality.

A "behavioral teratology" test of styrene has been mentioned in an abstract published in 1980 [121]. The abstract gives no information, except that a study has been performed, and no details have emerged.

2.2 HUMAN

2.2.1 Congenital malformation

A series of register-based case-referent studies of associations between congenital malformation and environmental exposures, particularly work-related, was begun in Finland in 1976 by the Institute of Occupational Health, Helsinki. Several publications since then have dealt with solvent exposure, and some specifically with styrene exposure.

The first report analyzed all CNS defects which occurred in the 9 month period June 1976 to March 1977[39;40]. Information on exposures to toxicants of 43 mothers who had had a child with a CNS defect, and their matched-pair referents (the previous delivery in the maternity welfare district), was obtained by interview and visits to the employers concerned. Two of the case mothers, but none of the referents, had been employed in the reinforced plastics industry, with exposure to styrene, polyester resin, organic peroxides and acetone. One child was anencephalic, the other had hydrocephaly, with ear, vertebral and rib defects. In this study, and all subsequent publications from this group, there were no qualitative or quantitative measurements of chemical exposure. No statistical analysis was attempted on this very small study population.

The author also reported the case of a mother of an anencephalic child who had been exposed during pregnancy to styrene, polyester resin and organic peroxides at home by her husband's working with reinforced plastic in kitchen repair work. The times of exposure during pregnancy, obviously critical in the case of anencephaly which is induced early in the first trimester, were not reported. The mother suffered from juvenile diabetes, which is a known risk-factor for congenital malformations, particularly neural tube defects.

Continuing with the same methodology, the study period was extended to 2 years (to June 1978). A total of 132 children born with CNS defects were reported to the Finnish Register of Congenital Malformations for this period. The final study series was 120 pairs, 5 cases being excluded by primary diagnosis of etiology, and 7 not participating [41]. The emphasis was turned to solvents in general, and 14 cases, but only 3 controls, were solvent-exposed, which is a statistically significant difference. Anencephaly was the most common defect, but 5 different classes of CNS defect were reported.

Most importantly, there were no additional cases with styrene exposure in this two year period, over and above the three observed in the first 9 month period.

These same data were further analyzed in a more detailed publication in 1980 [44]. Non-occupational exposures were excluded from this analysis, which reduced the number of solvent-exposed cases to 12 and the number of styrene-exposed to 2, the same 2 initially described. Exposure to organic solvents was still significantly more common in cases than controls. In addition, exposure at work to dusts was also more common in the study group than in the matched-pair referents.

Using the same Register and case-referent methodology, the group analyzed, in 1982, the relationships between oral clefts and organic solvent exposure during pregnancy [42]. For the 3.5 year period December 1977 to May 1980, the Register recorded 388 mothers of children with oral clefts, the final study population being 378 case-referent pairs. Exposure to solvents was significantly more common in cases than referents, there being 14 case mothers and 4 referents exposed. However, this was not the case for styrene, where there was one recorded exposure, both in cases and referents. In each case, exposure was due to construction of vehicle parts at home.

In 1983, the group presented a preliminary report [53] of their analysis of several classes of malformation, including CNS defects and oral clefts, with an extension of the study period to a total of 5 years, June 1976 to June 1981, and the inclusion of other exposures, in addition to organic solvents. The data on oral clefts was little different from that described above. There were 15 solvent-exposed cases and 5 exposed referents, one additional in each group. It is not possible to say if styrene was involved in either of these cases as information on individual chemicals was not presented. The numbers of mothers of children with skeletal or cardiovascular defects who had been exposed to solvents was the same as the number of referents.

The most interesting finding was for CNS defects. Unlike for the first 2 years, where there had been a significant excess of solvent-exposed cases (see above), solvent exposure during the subsequent 3 years was the same in cases and referents (6 exposed mothers in each group). Again, specific chemicals were not reported. The authors offered

two explanations for the discrepancy: either the initial association had been chance alone, or occupational health activities had reduced solvent exposures during the later 3 year period. They suggested that the frequency and severity of solvent exposure had declined during the study period, but no quantitative data were presented.

The group terminated their collection of data at the end of 1982, and reported a final analysis of 1475 case-referent pairs in 1986 [30]. Using questionnaire information, women were classified into 4 categories of solvent exposure: 0—"none", 1—"slight", <33% of the TLV (1981 ACGIH); 2—"noteworthy", about 33% TLV with peaks above the TLV; 3—"considerable", continuous exposure to > 66% but < 100% TLV; and 4—"heavy", >100% TLV. The authors give examples of categories, including several for 2, one of which was "Reinforced plastic manufacture in a boat building industry (main solvent styrene)". There were no women in category 4—"heavy".

The numbers of cases and referents with category 1, 2 or 3 exposures were reported for CNS, cleft, skeletal and cardiovascular malformations, but individual solvents were not given. The relative risk for maternal first trimester solvent exposure (categories 2) was 1.6 (95% confidence interval 1.0- 2.5) for all malformations pooled. A total of 12 women were specifically reported as being exposed to styrene (with acetone). Six at work in the reinforced plastics industry, 3 cases and 3 referents, and 6 outside of work during boat laminating, 4 cases and 2 referents. The types of malformations in these cases are not reported. There was no statistical analysis in the report. The number of styrene-exposed women in this study are too small to justify any conclusions.

The authors concluded that these data gave "limited evidence" that organic solvents, as a group, may be related to malformations, but that further studies are needed, and that "data on effects of solvent exposure during pregnancy is too sparse to allow for well-grounded scientific inference".

It seems clear from all these case-referent studies that the initial observation of two children with congenital CNS malformations after maternal exposure to styrene and other chemicals in the reinforced plastics industry was a chance observation. No other similar cases were found in a five year period. There was no evidence, at any stage, of an association between styrene exposure and any other class of malformation. There are several problems associated with the case-referent approach to reproductive epidemiology. In this case, a major limitation is the small size of the study population. Although these data provide no evidence for an effect of styrene exposure on rates of malformation, the power of the studies are limited. In addition, while the rate of reporting to the Registry is close to 100% for major unequivocal defects such as anencephaly and oral clefts, minor defects are less reliably reported and no conclusions can be drawn regarding these.

These studies provide some evidence that other organic solvents, as a group, may be associated with CNS and oral cleft defects, although the most recent data do not support the CNS association. It is difficult to assess the significance of studies of mixed exposures with no quantitation. There is, however, no reason to implicate styrene on the basis of these data.

Because of the original observations in their registry-based studies, the same group at the Institute of Occupational Health, Helsinki investigated, specifically, the whole population. However, the numbers were too small to permit further analysis, nor any firm conclusion. One confusing detail is that only one malformed child was reported for the women during exposure. We know from the original case-referent report of CNS defects [39] that at least two malformed children were born to occupationally styrene-exposed women during the study period. The authors do not discuss this, but the explanation is most likely that this current study did not cover all Finnish styrene-exposed workers.

A different group from the same Finnish Institute reported [38] on the risks of malformation for the children of members of the Union of Chemical Workers. Information on malformations was obtained from the Register, and that on branch of employment from the Union. For each chemical worker with a malformed child two control women were obtained from hospital discharge registers. The exact methods are not described. Exposure was assumed from whether the women had been working at the time of early pregnancy, again there were no actual exposures measured. For those female workers employed in the plastics industry the relative risk of malformation was 1(95% limits 0.2 to 5.5). Numbers were small: 9 exposed cases and 18 exposed controls. There was no mention of specific chemicals. These data show no evidence of an associationbetween working in the plastics industry and malformation.

In Sweden and Norway case-control studies have been performed on the outcome of pregnancy in female workers in the plastics industry[1]. The study period was 1973-1981; employment records were matched with national birth and malformation registers to identify cases of stillbirth or infant death, selected malformations, or low birth-weight. For each case, two controls from the same sources were matched for date of the birth, maternal age, and parity. Exposure data were from employers, with no individual measurements. Processing of styrene plastics was one of the exposure categories analyzed. The final study cohort

was 43 cases and 86 matched controls from Sweden, and 10 cases plus 20 controls from Norway, a total of 53 "triplets".

The analyses were performed by pooling all of the adverse outcomes of pregnancy together, so malformations can not be evaluated separately. The odds ratio for an adverse outcome was 0.8 (95% limits 0.4-1.6) for styrene exposure, suggesting no influence of styrene on pregnancy. This contrasts with exposure to PVC processing which had a ratio of 2.2(1.1-4.5). All further analysis in this report concerned PVC exposure.

2.2.2 Spontaneous abortion

A short, but controversial 1980 report by Hemminki and colleagues from the Institute of Occupational Health, Helsinki, Finland [35] raised concern about the incidence of spontaneous abortion among female chemical workers in general, and those exposed to styrene in particular.

Data on abortions were obtained from the Finnish National Board of Health computerized registry on in-patient discharges. For the years 1973-1976 there were records of 15,482 cases of spontaneous abortion, 71,235 induced abortions, and 193,897 births. These data were linked by social security number to membership of the Union of Chemical Workers, which gave information on the branch of chemical employment and date of joining. Only those women who joined the Union before hospital treatment were included. Two measures of the incidence of spontaneous abortion were calculated: the "rate" = no. spon. abn. x 100/ no. preg (spon. + induc. + births) and the "ratio" = no. spon. abn. x 100 /no. births.

There were 52 spontaneous abortions in Union members, at a rate of 8.54% and a ratio of 15.57%, significantly different from the 5.52% and 7.98% of the general population. Of the 5 branches of the chemical industry analyzed, styrene production and use had the highest incidences: 15.00% and 31.59%. Although these were also statistically significantly different from the general population, they were based on a total of only 6 spontaneous abortions in this branch. The incidences of spontaneous abortion in all women employed in industry and construction were 5.8% and 9.2%.

For all chemical workers, both the rate and ratio were higher than all Finnish women at all maternal ages, but the difference was greater in younger women, and greatest in the 15-19 year old group. Again, these are based on low numbers of Union members for individual age groups: 8 in the case of 15-18 year olds. There were no significant differences in age distribution of workers compared to the general population.

With regard to the possibility that styrene exposure may be related to spontaneous abortion, the major limitation of the study is the very small number of cases involved. In general, this was a very simple analysis of a very complex issue. The real incidences of abortion are influenced by many factors, including marital status, urban/rural residence, religion, smoking, diet and socio-economic status. None of these are examined in this study. Reported spontaneous abortions are generally not clinically distinguishable from induced abortion, and are therefore self-diagnosed. They are made up of true spontaneous abortions plus (illegal) induced abortions, which may be self-induced, or by a third party. Possible variation in abortion practices between chemical workers and the general population were not considered.

These differences . . . clearly do not support any increased risk due to styrene exposure at work.

This study was critically reviewed by Dr. T. Downs in an unpublished document sponsored by Daniel P. Boyd and Co., Leesburg, Virginia, on behalf of styrene manufacturers. The review reconstructed the raw data of the study and subjected it to a thorough statistical analysis. This showed that chemical workers had significantly larger rates and ratios of diagnosed induced abortions than all Finnish women. In addition, pregnancy rates and birth rates among chemical workers were significantly smaller than for all Finnish women. This shows that chemical workers were much less desirous of having a child than the general population, and therefore they seem likely to terminate a pregnancy, by a variety of means. Thus, the apparent excess of "spontaneous abortions" among chemical workers was more likely to be due to social factors than chemical exposure.

The same Finnish group have continued to monitor spontaneous abortions in relation to occupation. They have not confirmed their suggestion of an association of styrene exposure with increased spontaneous abortion. In 1984 they stated, in relation to the above study, "In this article, which was the first one in our ongoing study based on hospitalized spontaneous abortions, the diagnoses numbers taken for spontaneous abortions and for births were fewer than the ones used by us subsequently"[38]. They further analyzed spontaneous abortions among the members of the Union of Chemical Workers, according to Union membership during pregnancy, for the years 1973-1979. The first half of this period was that analyzed in their initial study [36].

For women involved in styrene production and use they found 9 spontaneous abortions during membership (rate = 6.8%, ratio = 10.2%), and 19 before or after membership (rate = 12.2%, ratio = 22.1%). Thus, the rate and ratio of spontaneous abortions during the period of putative exposure were half that at other times. These differences were not statistically significant at $p < 0.05$, but clearly do not support any increased risk due to styrene exposure at work.

This analysis also revealed two trends that suggested some form of selection (or bias) in the data. The rate of spontaneous abortion in all chemical workers did not increase with maternal age, which is abnormal. The rate of spontaneous abortions also decreased from 16% to 6% over the period 1973-1977. In relation to the observed reduced hazard during work with styrene, the authors concluded that the data need to be further analyzed with reference to the suspected selection mechanisms and actual exposures before hazard can be evaluated.

The last available report [58] from this group was published in 1985, and concerned the period 1973-1980. It was a case-control study, with 3 controls for each case. The study population was all female workers in the plastics industry who were Union members. Reproductive data were from hospital discharge and outpatient records. Information on exposures were from the women's occupational health physicians.

The odds ratio of spontaneous abortion was calculated by logistic regression and matched univariate analyses. The ratios for workers involved in the processing of cold and heated styrene were, respectively: 0.4(95% CI 0.1-1.2) and 0.6(0.2-2.3). In contrast, workers processing polyurethane had a ratio of 1.9, and all women employed in factories processing polyurethane had a ratio of 3.0, the latter being statistically significant. The authors concluded that neither the processing of styrene plastics, nor exposure to its thermal degradation products, was associated with spontaneous abortion.

Again, the number of spontaneous abortions in individual groups was small: 4 exposed cases, 17 exposed controls for cold styrene processing; 4 and 12 respectively for heated styrene. Because of the small size of the study population, the authors calculated that the likelihood of detecting a twofold risk was 38% for exposure to thermal degradation products, even less for specific plastics.

A study [32] by a different group from the same Finnish Institute was discussed above. Among 67 female lamination workers they did not find any difference in the incidences of spontaneous abortion during styrene exposure compared to before exposure. They did observe a reduced incidence of births during the exposure period, one cause of which was a higher number of induced abortions. This supports the assertion that working chemical workers do not wish to carry a pregnancy.

These data were re-analyzed recently by an independent epidemiologist [111]. Because a high rate of induced abortions complicates any study of spontaneous abortion, this re-analysis attempted to correct for this factor. It was shown that the highest spontaneous abortion ratio was in lamination workers during the exposure period, and the lowest was in the same group before exposure. However, it was stated that the numbers of events were too small to be of statistical significance.

2.2.3 Other outcomes of pregnancy

Two Russian reports describe various aspects of the health of women workers in polymer processing factories. A 1974 publication [133] dealt specifically with production of styrene polymers and the outcomes of 47 women who worked throughout pregnancy. It was reported that 18.3% of the women suffered "early toxicosis", 42.5% "late toxicosis", and 38.3% had "abnormal deliveries". None of these terms were defined and no further details were given.

The women came from two factories, and occupational conditions were quite diverse. Some women worked in an average of 3 ppm (13 $mg.m^{-3}$) styrene; some at 0.5 ppm (2 $mg.m^{-3}$) with an air temperature of 30-38°C; and some with a combination of styrene and dust exposure and heavy physical activity. In some cases women showed signs of styrene neurotoxicity.

This report provides little useful information. It is not possible to tell what adverse outcomes were suffered, nor whether any of these were related to exposures. Although it is reported that a group of non-exposed women from the factories was studied, no data on their pregnancy outcomes are given. Individual styrene exposures were not measured and the outcomes of pregnancy were not analyzed by occupational conditions, which were clearly very diverse.

The second report, published in 1983, concerned 287 women in polystyrene and polyethylene processing[63]. The rate of spontaneous abortion in these women was stated to be 11.3%, but no details and no control values were given. It was also said that toxicoses were more common in these women, but no data are reported. Premature rupture of the membranes and premature birth were 3 times more common in exposed than control women. The women often had multiple exposures, to styrene, ethylene oxide, carbon dioxide, heat and noise.

The significance of this report is also obscure. Actual numbers of pregnancies and adverse outcomes are not re-

ported. Styrene exposure was not measured but was clearly only one of several potential hazards.

A University of Cincinnati group has studied styrene exposure [54, 105], birth weight and length of gestation [56], and menstrual history (see section 3.2.1) in women in the reinforced plastics industries. In the study of pregnancy outcome, the final cohort was 229 exposed and 819 unexposed pregnancies giving live single births. Exposed women were employed, between 1974 and 1981, by 36 companies, mostly involved with open moulding, about half boat builders. Controls were unexposed women from the same or other local, companies.

Pregnancy outcome data was obtained by questionnaire, and work history from employer records. Individual styrene exposures were not measured. Mean styrene levels were calculated, based on 1500 samples taken in 28 companies, for 19 categories of work based on process, contact (direct or air), and product [54; 105]. Other exposures included: acetone, methyl ethyl ketone, methylene chloride, toluene and xylene. No levels are given, but those women with the highest styrene exposure also had highest exposure to other solvents. Exposures were analyzed by several methods: categorical (none/low[<30ppm in all months] /high); cumulative (ppmx days); and cumulative for critical periods (months and trimesters). Also, a subset was identified of the 50 highest exposed pregnancies in boat building, with an average exposure of 82 ppm styrene (and also high levels of other solvents) for at least one month. A series of covariates was also analyzed.

There was no effect of styrene exposure on length of gestation in any of the analyses. There was also no significant styrene dose-response trend in decreasing birth weight, again regardless of analysis method. The authors state that a 2% reduction would have been detected with 94% power. Factors that were significantly associated with reduced birth weight included: female sex, non-white race, cigarettes, length of gestation, lower gravidity, prior fetal loss or low birth weight.

For the 50 highest exposed pregnancies there was a 4% reduction in birth weight compared to unexposed controls, adjusted for all other factors. This did not reach statistical significance (P = 0.08), which the authors attribute to the small sample size. This birth weight reduction is comparable to that in smoking mothers, although smoking usually continues throughout pregnancy, while these exposures rarely extended beyond the 5th or 6th month. The authors suggest cautious interpretation of their data because styrene was used as a marker of overall solvent exposure, and exposure estimates were not specific to individual pregnancies, but were calculated from historical industrial hygiene measurements. This is a sensible conclusion to a careful study.

2.3 SUB-MAMMALIAN AND *IN VITRO* SYSTEMS

2.3.1 Chick

In studies reported in 1963 and '64 the effects of styrene injected into the yolk sac of chick eggs before incubation were described [67; 68]. These studies did not use an accepted "teratology" testing protocol and no control data are reported. The percentages of eggs that hatched with styrene exposure at doses of 2.3, 4.6 and 18 mg.egg^{-1} (46, 92 and 360 mg.kg[egg]$^{-1}$) were 95, 40 and 0% respectively. There were no reported abnormalities in surviving chicks. The authors suggested that their test was a useful indicator of toxicity, and that styrene had an intermediate order of toxicity.

A very similar order of magnitude of toxicity was described by Vainio, Hemminki and colleagues [120] who injected styrene into the air space of day 3 eggs. They report % hatch of 85, 30 and 0 for 20, 50 and 100 μmol.egg$^{-'}$ (about 2,5 and 10 mg.egg$^{-'}$). The equivalent figures for styrene oxide were 80, 30 and 0% for 1, 2 and 5 iimol.egg^{-1} (.12, .24 and .6 mg.egg^{1}). The chick embryo is stated to be more sensitive on days 0 and 1, but full data are not presented. The authors also state that doses of styrene and styrene oxide exceeding 10 and 0.1 innol, respectively, reduced viability, but the data presented show no effects until doses of 50 and 2, respectively.

It is difficult to interpret the malformation data, as they are reported. There are only average rates of malformation presented: 15% for 0.5-50 μmol.egg^{-1} styrene and 7% for 0.52.5 μmol.egg^{-1} styrene oxide. The authors state that attempts to define the dose-response relationships for styrene (10-50 umol) and styrene oxide (.5-2.5 umol) with administration on day 0 or 1 were not successful, but no data are presented. Ten different malformations are mentioned, but neither rates nor which chemical was involved are presented. It appears that malformed chicks were produced only with doses that were significantly embryolethal, but it is not possible to reconstruct the data from the information given. The illustrated malformed chicks were from doses that killed 70% of embryos.

The same group [52] examined the modifying action of the epoxide hydrolase inhibitor TCPO on the effects of styrene and styrene oxide administered to the air space on day 3 of incubation. Their data are most easily summarized as follows:

As in the previous study, malformed chicks were induced only at doses which killed a significant number of embryos. The authors concluded that TCPO enhanced the effects of styrene and styrene oxide, and therefore the ac-

tions of styrene must be mediated via metabolism to the epoxide. However, it is not clear if the combined effects are significantly greater than would be expected from the additive effects of the two chemicals. Thus, the evidence for enhancement is weak, particularly as the authors state that TCPO alone had a greater effect at higher concentrations (the data is not reported).

A group from India [110] administered styrene at 0.25-5 1.tmol.egg^{-1} into the yolk sac on day 3, 7 or 14 of incubation. The earliest stage was most sensitive, with the percentage of embryos dying increasing from 60 to 75% with dose. Although it is stated that control eggs were injected with vehicle solvent, there are no data presented on their rate of survival. There is no mention of malformation.

The levels of day 14 chick embryo liver haem and ALA synthetase were measured. In this case control values are given, but the styrene treatment is not explained. It is not clear when the eggs were exposed to styrene. Nor is it clear whether analyses were made on the livers of all, or just viable, chick embryos. This is important, given the high mortality rates. There was a dose-related decrease in ALA synthetase and increase in haem. The authors suggest that styrene blocks haem degradation (by binding to p450) which causes feedback inhibition of ALA synthetase, but no further data are presented.

4.4, respectively, for CNS cells, and 7.2 and 28.2 for limb bud cells.

The criteria for a positive response in this system are not well established. The original suggestion was that the 50% inhibitory concentrations should be <50 p.g.m1^{-1}, but one common current approach is to require that the effective concentration for inhibition of differentiation (IC50) is less than half that for cell survival (LC50). It is claimed that this indicates some specific action on developmental processes at concentrations not lethal to cells. The effect of styrene oxide on CNS cells is unusually negative in that treated cells are apparently able to differentiate even at several fold the LC50 concentration. In contrast, limb bud cell differentiation is inhibited at one quarter of the LC50. It has been claimed that a disparate response of the two cell types should also be a criterion of a positive response since it suggests specificity of action.

The validity of the micromass systems as predictors of developmental toxicity is currently being studied in inter-laboratory trials. No conclusion is possible at this time.

2.4 EVALUATION

There is one orthodox "segment II" study of styrene[73]. This is reasonably well reported, and of appropriate design for

	control	vehicle	tcpo .1umol	styrene 10umol	sty.ox .8umol	styrene +tcpo	sty.ox +tcpo
%dead	6	18	23	29	38	72	62
%malf	0	5	11	15	20	33	27

2.3.2 Other species

Pagano and colleagues [88] have described a test system involving the exposure of sea urchin gametes before, or embryos after, fertilization. They suggest that this may detect direct-acting mutagens. Sperm, eggs and embryos were affected by 10^{-4}M styrene or styrene oxide. The significance of these observations for mammalian toxicity is unknown. Many of the general comments below concerning the vulnerability of the chick embryo also apply here.

2.3.3 In vitro *systems*

A recent abstract [25] describes the effects of styrene oxide in the rat embryo 'micromass' cell culture system, which is increasingly popular as an *in vitro* screen for developmental toxicity. Concentrations of styrene oxide (p.g.m1^{-1}) that reduced differentiation and survival by 50% were 18.6 and

one species, but not the other. Rats were exposed to sufficient styrene (up to 600 ppm, or 300 mg.kg^{-1}/day, oral) to induce a decrement in maternal weight gain. The only significant effect on embryo/fetal development was a greater incidence of skeletal variations than in concomitant controls. Up to 1000 ppm styrene had no effect on maternal rabbits, but the highest dose used in the rabbit "segment II" test was 600 ppm. This exposure had no significant effect on embryo/fetal development, except, again, a higher incidence of skeletal variants than controls. The authors state that the incidence of skeletal variations in styrene-exposed animals of both species was within historical control values, but did not report the data.

There are five other studies of styrene effects in pregnancy in rats, mice and hamsters [20; 51; 97; 122; 130]. Each is defi-

cient in experimental design and reporting. It was reported that the incidence of embryonic, fetal and neonatal death was increased by inhalation exposure of rats to 0.35 to 11.6 ppm styrene [97], but these very low concentrations were accompanied by general maternal toxicity and the evidence for the reported effects is not convincing. Embryo/ fetal death was higher in 7 hamsters exposed to 1000 ppm styrene than in controls [51]. Maternal toxicity was stated to be "small", but no objective parameters were recorded.

Only one study has claimed that styrene exposure induces malformation. In a group of 13 mice exposed to 250 ppm styrene there were 3 malformed of 76 live fetuses, compared to 1 of 94 in controls [51]. There was no statistical analysis, nor is any possible from the reported data because litter values are not given, but this is unlikely to be a significant effect.

Continuous exposure of rats to 250 ppm styrene in drinking water through three generations had no consistent reproductive or developmental effect [8]. This exposure had an effect on water consumption and caused a small reduction in adult female body weight. Small decreases in neonatal survival and growth were seen in each generation of styrene-exposed pups. However, it is not clear if these were treatment-related since the exact parameter affected was different in each generation.

There has been a single study of the active metabolite, styrene oxide [112]. Exposure of rats to 100 ppm and rabbits to 50 ppm was lethal to some dams, but no malformation was induced. There were pre-implantation losses and fetal retardation, but in the presence of significant maternal toxicity.

There are no systematic studies of post-natal function after pre-natal exposure. Limited data from several studies do not suggest any major effect.

A report published ten years ago [39] described CNS malformations in two children of styrene-exposed mothers. This observation is still quoted as evidence of the developmental toxicity of styrene in humans *(e.g.*[108]) This is a misleading claim. Several larger and more powerful epidemiological studies from the same, and other, workers have failed to show that styrene exposure is associated with any increase in malformation [1; 32; 33; 38; 41; 42; 44; 53]. The obvious explanation for the initial observations is chance association.

A similar story has unfolded regarding spontaneous abortion. An early study suggested an association between styrene exposure and spontaneous abortion [36]. The study had some deficiencies of design and analysis. However, several subsequent investigations, from the same group, and others, of greater population size and more sophisti cated design, have not confirmed the association [32; 38; 58.] It is possible to conclude from these investigations that styrene exposure is not associated with a major increase in the occurrence of spontaneous abortion. But, because of the relatively small study population sizes in all these investigations, it is not possible to exclude the possibility of a small increase in risk.

All of these studies were carried out in Scandinavia, and none included measurements of individual exposure. It is not clear if the populations, or occupational health measures and exposures, are different from those in other countries.

There are little data on the potential effects of styrene exposure during human pregnancy on birth weight or premature delivery. One US study showed no effect of styrene exposure in the reinforced plastics industry on length of gestation, and no significant dose-response trend in decreasing birth weight [56]. This study suggests that high, direct, exposure to multiple solvents, including styrene at 80 ppm, may be associated with a reduction in birth weight of 4%, although this was not a statistically significant reduction in this small sample.

One Russian study suggests an increase in premature births, but the study report is seriously flawed, and exposures were to multiple potential toxicants, in addition to styrene. This study and a second Russian investigation also suggested an increase in "toxicoses" (presumably pre-eclampsia /eclampsia) with exposure to styrene, but no supporting data were reported.

The reported effects of styrene on chick embryo development [52; 120] are important because they are the basis for several published statements that styrene is a potential teratogen or developmental hazard. This is a misinterpretation of the data.

The effects of any chemical on chick development must be evaluated with caution. The chick egg is a closed system, one with little barrier to transport into the embryonic compartment, and of course there is no maternal surrounding environment. Added substances will generally remain for extended periods, and there is essentially no limit to the amount (concentration) of substance that can be added. Since all chemicals can be toxic, at some concentration, it is no surprise that virtually any chemical can produce an effect when added to the chick egg. This does not indicate a potential for developmental toxicity, but rather, simple general toxicity. Indeed, the chick egg has been proposed as an acute toxicity test (see above).

It is worth pointing out that any chemical would be developmentally toxic in mammals if a way could be devised to deliver the chemical to the conceptus without affecting the mother. Such a system would make the mammalian

embryo equivalently vulnerable as the chick. Thus, it is likely that the chick embryo is not intrinsically more sensitive than mammalian embryos, it is merely in a vulnerable position. Many hundreds of chemicals have been tested in various refinements of the chick egg system. Virtually all chemicals produce an effect. Some would suggest that chemicals be ranked according to potency. However, there is no known relationship between such potencies and mammalian developmental toxicity.

In the chick embryo, the production of specific malformations, at a significant incidence, in the absence of any general embryotoxic response, and at a concentration relevant to mammalian blood /tissue levels would be a reason to suspect specific developmental toxic potential. There is no evidence that either styrene or styrene oxide can induce such an effect. To the contrary, these compounds induce an unspecific spectrum of defects, only at concentrations which are embryolethal [52; 119].

The data on potential developmental toxicity are the most contentious of all those reviewed here. There is a lack of replicated, good-quality, experimental studies. However, it is clear from the combined weight of negative data on the induction of malformation that styrene should not be classed as a teratogen. Experimental data on other aspects of developmental toxicity is less clear, but there is little evidence that styrene is a specific developmental toxicant, that is, can affect development in the absence of maternal toxicity. Combined with the human experience, this suggests that it would be difficult to justify any further testing of styrene for developmental toxicity in experimental animals. Recently, an objective definition of "known to cause developmental toxicity" has been published, in relation to California's Proposition 65 [66]. In my view, styrene does not meet these criteria.

Early suggestions that industrial styrene exposure in pregnancy was linked with CNS malformations and spontaneous abortion have been clearly shown to have been spurious associations. It would prudent to continue to monitor the outcome of styrene-exposed pregnancies, and to keep exposure to a minimum, as for all xenobiotics. However, there is no compelling reason to propose major additional studies.

REPRODUCTIVE TOXICITY—FEMALE

3.1 ANIMAL

3.1.1 Estrous cycle

A brief Russian report published in 1957 [14] described "disturbances of a non-persistent nature in the reproduction cycle" in rats exposed to styrene in a sub-acute experiment at concentrations of about 3,500 and 7,000 ppm (15,000 and 30,000 $mg.m^{-3}$), and in a chronic experiment at 465 ppm (2,000 $mg.m^{-3}$).

The first experimental study of the effect of styrene on female reproductive function was reported by Izyumova from Moscow in 1972 [47; 48]. 60 female rats with strictly periodic estrous cycles, which had been checked for 2 weeks beforehand, were used. Groups were exposed to styrene by inhalation at concentrations of 50 $mg.m^{-3}$ (11.6 ppm) or 5 $mg.m^{-3}$ (1.2 ppm), plus controls (whether sham-exposed is not stated). There is no mention of the duration of daily exposure (assumed to be continuous), nor of inhalation conditions. Ovarian function was checked by vaginal smears to estimate length of cycles, over a four month exposure, and after one month of recovery. It is stated that 3840 smears were checked in total, but not when they were done. Since there were about 60 smears per animal and the project length was 5 months, there must have been an average of 3 /week/animal.

The results are not systematically presented, and are difficult to follow. It is stated that there was a significant increase in the number of leucocytes after 3 months of exposure to 11.6 ppm, plus 1 month of recovery, and for 1.2 ppm after 4 months plus 1 month of recovery. It is not clear if the effect was observed at both the end of exposure and after recovery, or only after recovery. Animals exposed to 11.6 ppm had a reduced weight compared to controls after 2,3,4 months of treatment and 1 month of recovery. Neither body weights, nor blood values are presented. At 4 months there was also a significant reduction in urinary hippuric acid to 40.77 *vs.* 62 mg% in controls. After recovery from 4 months of 1.2 ppm exposure, cholic acid was 1176 *vs.* 774 mg% in controls.

After exposure to 11.6 ppm for 1 month the length of the estrous cycle was 5.3 days *vs.* 4.2 in controls. There were no differences at 2 or 3 months for either exposure group. At 4 months the lengths were: 11.6 ppm = 5.8 days; 1.2 ppm = 5.3 days and control = 4 days. After recovery they were 7, 5.5, and 4.7 days respectively.

The "heat period", presumably the length of estrus, was reported as: 11.6 ppm = 2.8 days *vs.* 1.8 days in controls at 1 month; at 4 months: 11.6 ppm = 2.9, 1.2 ppm = 2.8, control = *1.7* days. After recovery: 5.5, 3.4, and 1.4 days respectively.

Neither the methodology nor the results of this study are adequately reported. It is not clear how the period of estrus was accurately measured. There are potential confounding factors, for example, what role might the reduced body weight have played? Was food consumption affected by styrene exposure? Clearly, the animals suffered general

toxicity caused by exposure, as manifested by the altered haematology and weight gain. There is no obvious explanation for the variation in effect with time: manifest at 1 and 4, but not 2 and 3 months, nor for the most significant changes being observed after 1 month of recovery. Despite these problems, the study can not be ignored since significant reproductive changes were apparently observed after supposedly low-level styrene exposure.

The same group published a repeat study in 1975 [132] with a very similar design, but a lower exposure concentration range of 1.2 ppm (5 $mg.rn^{-3}$, as before) and 0.2 ppm (1 mg. m^{-3}), plus controls. There were 67 exposed and 70 control rats. The exposure was for 4 months, followed by a recovery period. At 1.2 ppm the rats showed signs of general toxicity, including reductions in body weight and urinary hippuric acid, which were not observed at this concentration in the previous study. As in the previous report, details of methodology are poor.

Only the lengths of estrus periods are reported in detail. There were no statistical differences between control and 0.2 ppm exposed animals throughout the exposure period. At the higher concentration, the estrus period was significantly longer at 3 and 4 months: 2.6 days vs. 1.4 days and 2.3 days *vs.* 1.2 days, respectively, but not different at 1 and 2 months. This is not the same profile as was observed for this concentration in the previous study. After recovery, estrus was measured as: 3.4,2.7 and 1.1 days for 1.2 ppm, 0.2 ppm and controls.

Early suggestions that industrial styrene exposure in pregnancy was linked with CNS malformations and spontaneous abortion have been clearly shown to be spurious.

In an attempt to determine a mechanism for these effects, pituitary extracts were made from animals of each group at 4 months of exposure. These were injected into immature female rats, as a bioassay for gonadotropic activity. The uteri and ovaries of rats injected with styrene-exposed extracts weighed less than those treated with extracts from controls. No methodological details, nor any data, were presented.

Many manipulations are known to disrupt the estrous cycles of rodents. Even the regular taking of vaginal smears, or alteration of light cycles (were exposure chambers illuminated?), can cause profound effects. There is some inconsistency between this report and the previous study, and there are all the same deficiencies. Nevertheless, the second investigation appears to confirm an effect of styrene exposure on the length of the estrous cycle in rats, albeit with concomitant general toxicity. Again, the observation of the maximum effect after the recovery period is not easy to explain.

3.1.2 Ovaries

Bakhtizina and colleagues, from Ufa in the USSR, have studied the actions of styrene on the ovaries of the rat[3; 4; 5]. In an interesting study, they hemi-castrated groups of 10-12 mature rats by removing the left ovary and then observed the compensatory hypertrophy which takes place in the remaining contralateral ovary. Half of the animals were treated with 200 $mg.kg^{-1}$ styrene by gavage. It is not clear if this was a single administration, or daily dosing. Ovarian weights were taken from groups of animals at 7, 30 and 60 days after hemicastration. Ovaries of control animals increased in weight by 45%, 75% and 116% at these times, while those of treated animals were significantly lower at 7 and 30 days (22% and 30%), but not 60 days (111%). They also examined various structural features of the ovaries, including numbers and types of follicles, but report no effect of styrene treatment.

The mechanism of such compensatory hypertrophy is thought to be reduced feedback to the hypothalamus/pituitary, which causes enhanced gonadotropin secretion. The effect of styrene suggests some action on the function of the hypothalamus or pituitary. However, no data are presented on any general toxic effect of the styrene treatment used in this study, so it is not clear if this is a selective action.

The same group performed a morphometric and histochemical study of the ovaries of rats chronically exposed, by inhalation, to the "limiting concentration" of styrene[4]. The actual concentration is not reported, nor are any objective measures of its general toxic effect. They report the volume ratios (as % stroma) for corpora lutea, interstitial glands, and follicles at different stages of maturation, after exposure to styrene for 15, 60 and 120 days. There were significant changes at each time. Relative to controls, gland and follicle volumes were generally reduced at 15 and 60 days, but increased at 120 days.

NAD Diaphorase (a non-specific term, now little used, for any enzyme capable of oxidising NAD using any artificial electron acceptor) and G-6-P dehydrogenase were raised in ovarian structures from styrene exposed animals

at 15 and 120 days, but reduced at 60 days. 3-β-ol dehydrogenase and acid phosphatase had only minor changes.

Surprisingly, of the many published chronic/sub-acute and carcinogenicity studies of styrene which included routine histo-pathological examination of organs, few describe ovarian appearance. In one study life-long exposure to 250 ppm styrene in the drinking water had no effect on ovarian weight or morphology [8]. There was also no effect on fertility in this study, suggesting normal ovarian function.

In the study of styrene oxide reproductive toxicity 112 there was no reduction in fertility after 3 weeks inhalation exposure to 100 ppm styrene oxide, a treatment which was generally toxic, and lethal to some animals.

3.2 HUMAN

3.2.1 Menstrual cycle

In her 1957 and 1961 publications [14; 15], Bondarevskaya first suggested that industrial styrene exposure may be associated with menstrual dysfunction. She reported on women working in styrene concentrations of 4.7 to 30 ppm (20 to 128 $mg.m^{-3}$). During the first year of work 21.1% women had some menstrual dysfunction, a prevalence that reduced to 5.5% with 6 years of employment. In the 30 years since these suggestions, there have been numerous statements in the Russian literature that styrene exposure is associated with menstrual dysfunction. Those which are anything more than anecdotal are reviewed below.

Zlobina and colleagues [133] assessed the health of female workers in two polystyrene plants, in Kuskov and Zhilevsk. At Kuskov, they studied 238 exposed women, and a group of unexposed workers. The total number are not reported, but can be deduced to be about 117. They describe four types of working conditions, with styrene concentrations of: I: mean = 3 ppm (13 $mg.m^{-3}$), with standing and frequent movement; II: mean = 0.5 ppm (2 $mg.m^{-3}$), with air temperatures of 30-38°C; III: maximum = 0.7 ppm (3 $mg.m^{-3}$) , with dust, heavy lifting and constrained body position; IV: not stated.

They report that gynaecological problems were equally frequent in exposed and control groups, with the exception of vaginal inflammation (5.9% *vs.* 1.7%). The incidence of this varied with working conditions, and seemed to be correlated with physical activity, rather than levels of styrene exposure. Of the exposed women, 76 had a disturbed menstrual function (reported as 31.5%, actually 31.9% of 238), as did 19 control women (reported as 16.3%). Standard errors (or deviations, it is not stated) are reported for these figures, but how they were calculated is not clear. The highest incidence (38.7%) was in group II, which does not correlate with highest styrene exposure.

Vaginal smears were examined for 56 exposed and 28 control women. The most frequent defects were reported to be two-phase cycles with insufficient progesterone activity (39.3%) and anovulatory hypoestrogenic cycles (21.5%). The origin of these percentages is obscure. If they refer to exposed women, then control values are not given. It is stated that the greatest number of abnormal cycles was in group I, but no data are presented.

Exposed women also complained of gastro-intestinal and nervous disturbances. It is stated that a large fraction of exposed women had dystrophic changes in upper respiratory tract mucus membranes and chronic tonsillitis. They also had impaired blood clotting.

Many fewer data from the Zhilevsk factory are presented. Of 163 styrene exposed women, 37 (stated as 23.1%, actually 22.7% of 163) had menstrual function disorders. No control values or exposure data are given. Vaginal smears were examined for 19 exposed women. They showed similar abnormalities to those described above, but no data are presented.

The same author also reports [132] on 110 exposed and 231 control women from a plastics factory. Exposure is stated to be to "a constant low concentration of styrene", but no further details are given. There were no differences between exposed and control women for the incidence of gynaecological problems, with the exception of inflammation of the uterus and fallopian tubes (12.7% *vs.* 4.7%). Menstrual disturbances were more common in exposed women (29.1% *vs.* 9.1%). Symptoms reported were: disturbances in "rhythm"; oligodysmenorrhea, hyper- and hypomenorrhea.

Loseva and colleagues [63] reported on women employees in polymer processing plants who were exposed to styrene, ethylene oxide and carbon dioxide (plus other unmentioned chemicals), at concentrations which are stated to exceed acceptable limits by 1.5 to 3 fold, but actual values are not reported. The health of 287 women was evaluated, but the numbers of exposed and unexposed is not given. The women complained of a wide variety of adverse health effects, including nervous system dysfunction.

Gynaecological problems were more common in exposed women (36.1% *vs* 12.3%), and included inflammations of the vagina and uterus. Menstrual disturbances were also more common (33% *vs* 8.6%). Specifically, dysmenorrhea (21.6% *vs* 4.7%) and hypermenorrhea (11.1 *vs.* 2.1) were of increased frequency in exposed women. No other details are reported.

Other Russian researchers who have reported apparent increases in menstrual disorders in women occupationally exposed to styrene (concomitantly with other chemical or physical agents in all cases) include: Pokrovsy in 1967 93; Bobrova in 1977 [12] and Gorobets in 1984 [24].

Only two non-Russian studies have addressed this issue. In Harkonen and Holmberg's study [32] of 67 Finnish female lamination workers and their matched controls (see sections 2.2.1 and 2.2.3) they examined: duration of menstruation; incidence of irregular menstruation; and changes in menstruation during the exposure period. In no case was there any difference between exposed cases and referents.

By far the most comprehensive study of menstrual function and industrial styrene exposure is that of Lemasters *et al.* [55] who studied workers from 36 US reinforced plastics companies (see section 2.2.3). The final study population was 174 exposed and 449 unexposed women. The two groups did not differ in age; parity; race or income. Menstrual histories were taken by telephone questionnaire, and were obtained before the exposure information, which was from historical measurements and in-factory observation. Exposures were categorised as being of two types: open-mould manufacture of boats, trucks and car parts (51 women), with an estimated weighted mean exposure of 52 ppm styrene, and all others (123 women), with mean exposures of 13 ppm.

Using chi-squared analysis, it was shown that exposed and unexposed women did not differ in the incidences of: severe dysmenorrhea; intermenstrual bleeding; secondary menorrhea; menstrual blood clots and hypermenorrhoea. Multiple logistic regression analysis showed that exposure to styrene was not associated with menstrual disorders in any exposure model. Risk factors that were associated with disorders included: age; nulliparity; smoking; and chronic illness. The authors estimated that the study had a 85 - 90% power to detect a doubled incidence of these menstrual dysfunctions.

This study also provides background data which may help to put the Russian studies in perspective. The authors review those factors known to be linked with menstrual disorders. They include: age; extreme weight range; parity; liver disease; smoking; thyroid abnormalities; stress; exercise; use.of IUD's; marital status; and exposure to various drugs, including oral contraceptives. Most of these risk factors were included in the multiple logistic analyses of Lemasters *et al.* [55]. As far as is possible to tell from the reports, none of the Russian studies took any of these factors into consideration.

The report gives the "control" incidences for various menstrual disorders for the study group of 449 unexposed women (which were not different in working and non-working women). The overall prevalences were: dysmenorrhoea 14%; intermenstrual bleeding 16%; menorrhea *7%;* menstrual blood clots 40% and hypermenorrhoea 31%. It is interesting that most of the prevalences in the exposed women in the Russian studies do not exceed these values, while the incidences in unexposed women are reported to be considerably lower.

3.2.2 Ovaries

No available data.

3.3 EVALUATION

There are no reports of an adverse effect of styrene on female fertility in experimental animals, but no study has been designed specifically to examine the possibility. Female rats exposed to 250 ppm in drinking water *in utero* and throughout life did not show any impaired fertility [8]. Exposure of female rats to 100 ppm styrene oxide for three weeks by inhalation also had no effect on fertility [112].

Two Russian studies [14; 132] suggest an increased length of the estrus cycle in rats chronically exposed to 1.2 or 11.6 ppm styrene, but no conclusions can be drawn. The effect was inconsistent during exposure, and was maximal one month after cessation of exposure. These very low levels of styrene were reported to cause reduced body weight. Many factors known to affect the estrus cycle in rodents were not considered.

There are only limited data on the potential effects of styrene on the ovaries. Russian studies suggest an effect on rat ovarian morphology and enzymatic activity with inhalation exposure at "limiting" styrene concentration, and on compensatory ovarian hypertrophy after hemi-ovariectomy at 200 $mg.kg^{-1}$ [3; 4; 5]. General toxicity was poorly reported and monitored in these studies.

It is difficult to assess the overall significance of the Russian reports that describe an effect of styrene exposure on women's menstrual cycles. They are easy to fault. They all suffer from inadequate presentation; poor data; lack of appropriate controls and analysis; confounding exposures and conditions; etc. However, they do show a consistent pattern of more menstrual disturbances in women exposed to styrene than in unexposed groups. In contrast, the single US study, which is of good quality, and a small Scandinavian investigation show no such effect.

It is possible that differences in exposure explain this disparity. Styrene exposures were reported to be generally about 10-fold lower in the Russian factories, than in US facilities. However, the Russian women were reported to

suffer neurological symptoms and actual exposure may have been much higher. In general, retrospective menstrual histories are unreliable and even data collected prospectively is difficult to interpret [33]. There are some other data which suggest an effect of styrene on CNS control of reproductive function (see section 5).

REPRODUCTIVE TOXICITY—MALE

4.1 ANIMAL

4.1.1 Fertility

Few studies provide information on the fertility of styrene-exposed male animals. The three generation rat study described in section 2.1.1 showed no infertility effect of continuous exposure to 250 ppm styrene in drinking water throughout life. In a dominant lethal study of styrene oxide [21], mice were treated i.p. with a single dose of 250 $mg.kg^{-1}$. Over a three week breeding period with untreated females, there were no differences between treated and control males in the number of: females pregnant; corpora lutea; implantations; live and dead fetuses. Thus, there was no effect on fertility, nor any dominant lethal effect (post-meiotic stages only tested).

4.1.2 Testes

Some routine histopathology studies have examined the testes of styrene treated animals. Following subacute styrene exposure no overt effects on testis morphology were noted in these species at these maximum doses (data summarized in [34]): rat: 2000 ppm by inhalation, 7 hours/ day, 5 days/week, for 5 to 7 months; 3160 $mg.kg^{-1}$ oral, 5 days/ week for 7 weeks; guinea-pig: 2000 ppm by inhalation, as above; rabbit: 2000 ppm by inhalation, as above; mouse: 681 $mg.kg^{-1}$ oral, as above; rhesus monkey: 1300 ppm, 7 hours/day, 5 days/week for about 13 months.

Some testicular pathology was noted in a chronic study of styrene in the rat[50]. Male rats were exposed by inhalation (6 hours/day, 5 days/week) for 18 months with observation to a maximum of 24 months. Low dose concentration was 600 ppm throughout, high dose was 1200 ppm for 2 months, 1000 ppm for the remainder. There was reduced body weight at both doses. At interim kill times of 6 and 12 months there were focal microscopic lesions of the testes at both doses. However, changes were restricted to a particular region which suggested that the cause was physical trauma due to reduced size of the scrotum, rather than a direct action of styrene. Spermatogenesis and morphology were normal throughout the rest of the testis and epididymis.

In the rat multi-generation study of 250 ppm styrene in the drinking water [8] there was no effect on testicular weight (relative and absolute) or histology following exposure *in utero* lactation and throughout adult life.

Styrene has been tested in the mouse sperm morphology test [104]. Although this test is designed to evaluate mutagenic potential, it can provide some information on reproductive effects. The compound was administered to male C3Hx C57 F_1 mice by inhalation at 150 and 300 ppm (6 hours /day for 5 days) or by intraperitoneal injection at 175, 350 and 700 $mg.kg^{-1}$ for 5 days. There was a slight body weight loss in the 700 $mg.kg^{-1}$ group during treatment, but no changes in testicular weight at the assay times of 3 and 5 weeks post-exposure (actual data not reported). Styrene treatment had no statistically significant effect on the numbers of abnormal sperm. Sperm counts and testicular morphology were not reported.

A recent report [118] describes the effects of oral administration of 200 or 400 $mg.kg^{-1}$ styrene, 6 days/week for 60 days, to male rats. Styrene was "of the highest purity" but no value is reported. This was a small study, with 6 rats per group, including vehicle-treated controls. One testis was fixed for pathology, the other homogenized for enzyme assays. Spermatozoa were obtained by mincing epididymides. It is stated that there were no deaths, nor any effects of treatment on body, testicular or epididymal weights, but the data are not presented.

At the higher dose, there was a reduction in epididymal sperm count (5.3 *vs.* $8x\ 10^6$, volume units not reported) and there were changes in testicular morphology including degenerating, "shrunken" tubules and germ cell loss from tubules. The changes, are not described in detail and the number of animals with such changes, and the consistency of observations in a single organ are not discussed. The lower dose was without any statistically significant effect.

All enzymatic activities measured were affected by the high dose: sorbitol dehydrogenase and acid phosphatase increasing; LDH, GGT, G6PDH and β glucuronidase decreasing. The authors state that these changes are compatible with some effect of styrene on germ cell maturation, which is in turn compatible with the histological changes.

As discussed, this study has deficiencies and should be considered preliminary. It appears to show an effect of oral administration of 400 $mg.kg^{-1}$ styrene for 60 days on testicular function and morphology, without a concomitant effect on body weight. However, the same group have previously reported a study of very similar design in which the same dose of styrene caused various changes in liver

enzymatic activities, and hepatic focal necrosis "'. In that study, the source and purity of styrene is not mentioned, but the dosing regimen was the same as the testicular study, except that treatment began at an earlier age (110 *vs.* 225 g body weight) and lasted 100, rather than 60, days.

Again, it is reported that the treatment was without overt toxicity or effect on body and liver weights, although data are not shown. Increases in serum GOT and GPT, and small areas of focal necrosis were observed at 400, but not 200 $mg.kg^{-1}$. This suggests that the exposure to styrene that caused testicular changes may also have been mildly hepatotoxic. However, the hepatic effects were reported after a longer exposure period, and no liver-related parameters were measured in the testicular study, so no definitive conclusion can be made. It is possible for liver changes to induce secondary action on the testes, by increasing hepatic metabolism of testosterone, for example. However, it does not seem likely that the reported liver effects of styrene are in any way related to the kinds of testicular actions suggested above.

4.2 HUMAN

A 1976 report [78] from the USSR discusses 143 workers aged 20 to 45, with 1 to 10 years service in a reinforced plastics factory where they were exposed to styrene and phenol, formaldehyde, aniline, epichlorohydrin and dust, at levels which exceeded limits by 1.5 fold. About 40% (58 men) complained of sexual problems including reduced libido and erection/ejaculation defects. The severity seemed to be correlated with length of service. Semen samples were examined, and in about a third, the volume of ejaculate was reduced (<2m1) and in one half, the viscosity and time to liquefaction was increased. In one third of the men their sperm count was at the low end of the normal range, and was lower than normal in the remainder. There was an increase in immobile sperm in 23% of men. There was an increase in the urinary excretion of 17 ketosteroids in 33 of the men. These men also complained of a wide variety of nervous and psychological problems, some of which were confirmed by objective measures. The authors concluded that the sexual problems were most likely the result of CNS dysfunction.

It is not possible to draw any conclusions from this study regarding the effects of styrene on male reproductive function. Styrene was only one of several toxicants to which the men were exposed, and relative exposure levels are not given in the report. The report is very brief, there are no actual data values, and no control observations.

A 1988 publication describes semen quality in workers from a Danish reinforced plastics factory which manufactured windmills ". Semen and blood samples were collected from 25 men within three weeks of production at the plant being halted. Workplace styrene median concentrations were 68,84 and 128 ppm (294,362 and 552 $mg.m^{-3}$) with peak levels generally two fold higher. The Danish TLV for styrene is 25 ppm. Acetone was measured simultaneously and median values were 0.3 to 0.6 of the TLV of 250 ppm.

As a control group, the study used semen and blood samples from men on their initial visit to an infertility clinic. When these samples were taken, the etiology of the couples' infertility was unknown. These men were matched for age with the exposed men, but not for any other confounding factors. Only a single sample was taken from exposed and control men.

The two groups did not differ in: serum LH; serum FSH; semen volume and sperm count. Semen from the exposed group was apparently superior to the control group for % live sperm and % motile sperm (the authors do not state how they distinguish immotile live from dead sperm). In contrast, the % normal sperm was significantly lower in the exposed group. In particular, head shape abnormalities were more common, mid-piece and tail defects were not.

As the author acknowledges, the choice of control group is a problem in the study. This group would be expected to have a poorer semen quality than the general population. About half of all cases of infertility are male-related, and a major portion of these are reflected in various decrements in sperm parameters. This probably explains the inferiority of the control samples for % live and % motile sperm. It is possible that the same phenomenon may mask other changes in the exposed group, relative to the general population, and it suggests that the increase in abnormal sperm head shapes in exposed men would have been greater compared to appropriate controls.

Sperm parameters are known to vary significantly between semen samples from the same person at different times, and multiple samples are superior to the single samples taken in this study. However, sperm head morphology is one of the least variable measurements.

The workers were exposed to heat (no details given), as well as styrene and acetone. It is not possible to exclude interactions, or the other factors individually, but styrene appears the most likely potential toxicant. Heat has not been reported to affect sperm morphology, and acetone

was present at lower concentrations, relative to accepted limits, than styrene. In animal studies, an effect on sperm head morphology has been correlated with carcinogenic potential. In the only published study, styrene was negative in a mouse sperm morphology test (see section 4.1.2)

4.3 EVALUATION

The available data on the male reproductive toxicity of styrene are inadequate to draw any firm conclusions. No study has been designed specifically to examine potential effects on the fertility of experimental male animals.

There was no effect on male fertility of rats continuously exposed to 250 ppm styrene in drinking water [8]. Fertility of male mice was not altered during the three weeks following a single dose of 250 mg.kg^{-1} styrene oxide[21]. The large reserve in male reproductive capacity of rodents is well known, however, so these may not be sensitive measures of testicular toxicity.

General studies of the chronic toxicity of styrene in several species, including the monkey, have not revealed any testicular pathology at concentrations up to 2000 ppm[34]. The only evidence of testicular toxicity comes from a recent study in the rat [118] which suggests that treatment with oral styrene doses of 400 mg.kg^{-1} for 60 days decreases sperm count and induces some testicular pathology. This treatment was stated to be without general toxicity. However, a previous publication[117] from the same workers reported hepatic toxicity following a very similar experimental design. Liver function was not evaluated in the testicular study. It is very unlikely that the hepatic effects reported could have caused secondary testicular effects, but it is possible that the testis and the liver are affected at similar exposure levels.

One study of men occupationally exposed to up to 130 ppm styrene showed an increase in sperm head shape abnormalities, but not other sperm parameters [49]. The study was small, poorly controlled, and used only single semen samples. The exposure of mice to up to 300 ppm or 700 mg.kg^{-1} for 5 days did not cause sperm head shape abnormalities.

There are current proposals, from India, to use a styrene-based polymer as a male contraceptive agent. Styrene maleic anhydride injected into the vas deferens is an effective and reversible contraceptive in rats and monkeys [27; 109]. The mechanism of action is thought to be local lowering of pH, and occlusion. This polymer also inhibits the motility of human spermatozoa *in vitro* [113]. The potential toxicity of leached styrene monomer has not be addressed.

Although some reports have claimed an effect of styrene exposure on testicular and sperm morphology, the bulk of current data do not suggest that styrene is a specific testicular toxicant.

ENDOCRINE

5.1 ANIMAL

5.1.1 Hypothalamus/pituitary

An Italian group has published a series of studies on styrene effects on dopamine levels in various areas of the brain [74; 75; 76; 88]. These are relevant to the same group's observations on pituitary function in styrene-exposed workers (see below). In summary, inhalation exposure of male rabbits to 1500 ppm for 12 hours/day for 3 days causes a significant decrease in tubero- infundibular and striatal dopamine content, and an equivalent increase in homovanilic acid. Norepinephrine levels and dopamine turnover and receptors were unaffected [75]. A similar effect could be induced by i.p. administration of phenylglyoxylic acid, phenylglycine [76] and mandelic acid [99]. Dopamine content recovered after 3 weeks and neither synthesis nor catabolism were affected at any time [76]. Dopamine was shown to non-enzymatically condense *in vitro* with glyoxylic acids to form isoquinoline compounds [74].

These observations are different from those of an Indian group who have studied the effects of daily oral administration of 1000 mg.kg^{-1} styrene to rats for 14 days [130]. They found no effect on dopamine levels, but increases in norepinephrine and serotonin, and in striatal dopamine receptors. They also found similar effects of styrene oxide at 50 mg.kg'$^{-1}$ [45; 131].

Russian studies have suggested an effect of styrene exposure on the morphology of the hypothalamus/pituitary [3; 95]. Chronic exposure of rats to 23 ppm (100 mg.m^{-3}) styrene, 4 hours /day, caused morphological changes to the median eminence of the hypothalamus and pituitary.

5.1.2 Adrenal

A Russian group has published a series of reports on the effects of styrene exposure on the morphology and enzyme histochemistry of the adrenal of rats [96; 102; 103.] Various changes were observed within one month of inhalation exposure of rats to 23 ppm (100 mg.m^{-3}) styrene, 4 hours/day. Corticosteroid metabolism was significantly affected, 11-B-hydroxylase being inhibited [96; 102] and 3-β-steroid dehydrogenase stimulated [102], as measured histochemically, but serum levels of corticosteroids were not reported.

Although severe adrenal dysfunction can affect reproduction, the significance of these observations is unknown.

5.2 HUMAN

In a brief report [7], Bashirov describes some aspects of endocrine function in 44 women and 26 men exposed to sty-

rene, "divinyl", and other chemicals in a Russian synthetic rubber factory. Based upon levels of urinary hormones, the author concludes that the order of sensitivity of endocrine glands is adrenal thyroid pancreas. In addition, he states that the effects are most likely to be central in origin since all workers with endocrine effects simultaneously demonstrated signs of CNS toxicity. There are no details of chemical exposures, sample collection, or control groups.

A similar, but more detailed, study has been performed on male workers in a Dutch synthetic rubber facility [128]. Urinary 17-oxo and 17-keto steroids were measured in 25 exposed and 26 non-exposed workers from the same factory matched for age and some socio-economic factors. Some workers had increased excretion of amygdalic acid, indicative of styrene exposure, but the author reports that other objective findings which might point to inhalation of styrene were absent. The excretion of measured steroids was not statistically different in the two groups of workers.

A group from Parma, Italy, has studied the neuroendocrine effects of styrene (see above), and in particular, the dopaminergic control of pituitary function [2; 77]. Pituitary secretion of prolactin (PRL) is directly inhibited by dopaminergic tuberoinfundibular neurones, whereas the control of secretion of growth hormone (GH), TSH, and the gonadotropins LH and FSH is more complex, involving the hypothalamic peptides: LH-RH; TRH and somatostatin.

Serum hormone levels were measured in 30 women exposed to styrene in two reinforced plastics factories, and in 30 age-matched unexposed female workers from the same area [77]. Urinary excretion of mandelic acid (MA) and phenylglyoxylic acid (PGA) suggested mean daily styrene exposure of 130 ppm (range 65-300). Serum PRL levels in exposed women were twice that of controls and were significantly related to MA + PGA, but not duration of exposure. Overall, TSH levels were not elevated in exposed women, but there was a significant relationship with MA + PGA. Serum GH was elevated, but the correlation with MA + PGA is not reported. Serum LH and FSH were not statistically different in the two groups. The authors speculate that a styrene metabolite may compete with dopamine for storage capacity.

In a follow-up study the effect of an exogenous TRH challenge on serum PRL was examined[2]. The study group (probably from the same location as above, although not explicitly stated) was 16 styrene-exposed women and 16 age-matched controls. Serum PRL was measured before and after an injection of TRH. Urinary MA and PGA were also measured. The exposed group had significantly higher basal PRL levels, but the increase was small (unlike the previous study) and within the range of normal values. [Absolute PRL serum concentrations are at least 3-fold higher in this, compared to the previous study, which the authors do not discuss].

TRH-stimulation of serum PRL was much elevated in exposed women, at all times after injection. There was a significant relationship between maximum stimulated PRL level and urinary MA + PGA, and a slightly better correlation with PGA alone. Baseline PRL was not correlated with MA + PGA (unlike the previous study). Duration of styrene exposure was not correlated with any PRL value.

Three exposed women were re-evaluated after a 3 month no-exposure period, and each had a more normal response to TRH. The authors report that the exposed group had high scores on a rating scale for depression, and 50% had menstrual or sexual disturbances. No details are presented, nor are any control data.

The significance of these observations is unclear. While it is reasonable to assume that decreased hypothalamic dopamine may play a role in hyperprolactinemia, these data do not provide any information on hypothalamic dopamine levels. There is no known function for TRH stimulation of PRL and the doses of TRH used here were supraphysiological. In addition, there are inconsistencies between the two studies.

5.3 EVALUATION

Rabbits exposed to 1500 ppm styrene for three days show changes in brain dopamine levels which might affect the function of the hypothalamus and pituitary, and reduce the secretion of prolactin [74; 75; 76; 99]. Some aspects of these changes can be induced by the stable acid metabolites of styrene. There is weak evidence for styrene-induced morphological changes in the hypothalamus and pituitary [3; 95].

Observations on women occupationally exposed to up to 130 ppm styrene also suggest an effect on pituitary secretion of prolactin [2; 77]. Hyperprolactinemia can be associated with menstrual dysfunction. The supporting evidence is not strong, but is of interest in the light of repeated suggestions of menstrual dysfunction in styrene exposed women.

ABSORPTION, DISTRIBUTION AND METABOLISM

There is a large literature on these aspects of styrene toxicology, and it has been well reviewed recently [13]. As a very brief summary: in experimental animals and man, styrene is well absorbed and rapidly metabolised following all modes of administration,-The major route of excretion is the urine, with mandelic and phenylglyoxylic acids as the major metabolites. Dose-dependent pharmacokinetics are observed, with reduced metabolism at higher doses. Styrene distributes widely into body organs. Adipose tissue can act as a reservoir for styrene, but not for extended periods. Chronic styrene exposure induces its own metabolism.

The metabolism of styrene is well studied [13]. The major pathway is: 1) activation by cytochrome(s) p450 to styrene oxide; 2) conversion of styrene oxide to styrene glycol by epoxide hydrolase(s); 3) dehydrogenation of styrene glycol to mandelic acid, and further to phenylglyoxylic acid. However, the complete picture is much more complex, with multiple competing pathways, including conjugations with glucuronide, glutathione, glycine and sulfate. Recently, stereochemical aspects have been considered n.

The occurrence of target organ toxicity depends upon the pharmacokinetics of the ultimate toxicant(s) in that organ, or a sub-population of susceptible cells. For any chemical this is a complex phenomenon dependent upon distribution of parent compound and metabolites into and out of the organ, and upon rates of synthesis and degradation of the ultimate toxicant(s) in the organ.

In the case of styrene this is particularly complex. There are multiple isozyme forms of the enzymes of activation *(e.g.* p450) and inactivation *(e.g.* epoxide hydrolase and glutathione-S-transferase), with differential expression during development and in different organs and different sub-cellular fractions. It is generally thought that styrene oxide is the ultimate toxicant, being a reactive electrophile capable of interacting irreversibly with nucleophilic centers in proteins, nucleic acids, etc. However, it has been suggested that stable acid metabolites may be the toxic agents in certain responses to styrene *(e.g.* CNS effects, see 5.1.1).

A complete reproductive and developmental toxicity risk estimation would require consideration of all of these factors for reproductive organs and for the conceptus, of experimental animals and man. I doubt that all of this information is available for any chemical, and it certainly is not for styrene. In the remainder of this section I will briefly review the relevant available data.

6.1 ANIMAL

6.1.1 Distribution—transplacental

Styrene is a small, uncharged lipophilic molecule and the first principles of placental transfer would suggest that it would pass freely across the placenta by passive diffusion.

A very brief Russian report published in 1974 describes placental transfer of styrene on days 18 to 21 of pregnancy in rats [134]. Animals were exposed by inhalation to 3.6 or 10 ppm styrene (15.5 and 43.2 $mg.m^{-3}$) and after 2 hours maternal and fetal blood and amniotic fluid samples were analyzed for unchanged styrene (no data on metabolites). At 3.6 ppm, concentrations ($i.tg.m1^{-1}$) were: maternal blood, 1-3; fetal blood 1-2; amniotic fluid, 1-2, and at 10 ppm they were 11-12; 8-9 and 2-3 respectively.

Thus, tissue levels increased up to 10-fold for a 3-fold increase of exposure concentration, suggesting saturation of some aspect of disposition. Fetal blood concentration was slightly lower than that of the mother. It is possible that this is because the fetal compartment had not reached steady state styrene concentration. The authors report that adult blood levels were constant after one hour of exposure, but present no data on the kinetics of the fetal compartment.

In a paper concerned with intra-litter variation following *in utero* exposure in chronic toxicity and carcinogenicity studies [26], there are some data on fetal styrene concentrations. Five Sprague-Dawley rats were exposed to 2000 ppm styrene for 5 hours on day 17 of pregnancy. Litter mean values for whole fetal tissue styrene concentration varied between 35 and 65 $\mu g.g^{-1}$. Maternal blood and tissue concentrations were not measured.

A similar, but slightly more extensive, study was published in 1985 [129]. Sprague-Dawley rats were exposed for 5 hours on day 17 of pregnancy to 1000 ppm (6 animals) or 2000 ppm (5 animals). Maternal blood and whole fetal tissue was analyzed for unchanged styrene. Mean styrene levels ($1\text{-}\mu g.g^{-1}$) were: maternal blood, 36 and 89; fetal tissue, 17 and 46, at 1000 and 2000 ppm respectively. (Note that the fetal level at 2000 ppm is in good agreement with the study above; 46 cf 35-65). Previous studies by the same group had shown adult tissue (heart, lung, liver, spleen, kidney and brain) levels after these same exposures, 1000 and 2000 ppm for 5 hours, to be 49 to 93, and 184 to 382 $\mu g.g^{-1}$.

Thus, all maternal and fetal styrene levels increased by slightly more than 2-fold with a doubling of exposure concentration. Fetal styrene levels were only about half that of maternal blood at both exposure concentrations. Also, fetal styrene levels were only one quarter to one third of the lowest adult tissue levels (at least of those organs analyzed here). The study provides no information on metabolite levels, nor on kinetic aspects, save that steady state adult blood levels were not reached after 5 hours. The report describes significant inter- and intra-litter variations in styrene levels.

6.1.2 Distribution—reproductive organs

Tissue levels of styrene in rats following a single oral dose of 20 $mg.kg^{-1}$ peaked at 2 hours for most organs [92]. In males, the peak level in the testes was 3.6 $\mu g.g^{-1}$, which was comparable with brain and muscle, but considerably lower than kidney [46], liver [13] and pancreas [10]. In females, a similar profile was observed with ovarian levels of 2, compared to 24, 7 and 6 $\mu g.g^{-1}$ for kidney, liver and pancreas respectively. Testicular and ovarian levels fell to about 0.2 $\mu g.g^{-1}$ by

12 hours, and <0.01 by 24 hours.

Following intraperitoneal injection of 3.3 mmol.kg^{-1} to mice, the highest total styrene (plus metabolites) level in the testes was observed at the first time point of 30 minutes[60]. This was less than half of the levels found in subcutaneous adipose tissue, pancreas, liver and kidney. The metabolite, styrene glycol, was also detected at this time, at levels close to that in liver and pancreas, but about half that in lung and kidney. However, the level of conjugated styrene glycol was low in testes at all times measured, suggesting low levels of conjugating enzymes. There was a linear increase in testicular styrene and styrene glycol levels at 2 hours within the dose range 1.1 to 4.9 mmol. kg^{-1}.

The levels of styrene oxide in mouse organs after exposure to styrene has been reported [61]. For all organs, tissue levels of styrene oxide were less than 10% of the concentration of parent compound. Reproductive organs were not examined. There was some correlation between the p450 enzymatic activity of organs and the levels of styrene oxide.

6.1.3 Metabolism—reproductive organs and the conceptus

Styrene oxide is commonly used as a model substrate in studies of epoxide hydrolase and glutathione-S-epoxide transferase activities, and the tissue distribution of these enzymes is well studied.

In rat and mouse, the testis is relatively rich in epoxide hydrolase [79], indeed it has the highest specific activity of any organ in NMRI mice. The hamster testis has much lower activity [80]. In Sprague-Dawley rats the testis is second only to the liver in specific epoxide hydrolase activity, while the ovary is not quite as active, but still has significant activity, on a par with kidney and lung [79]. It has been suggested that steroids are the endogenous substrates for gonadal epoxide hydrolase [11].

Glutathione-S-epoxide transferase activity in rat ovaries is about 70% of that in liver, and exceeds it during pregnancy and lactation [72]. Rat testicular activity is about 60-70% of liver in adult animals, and even higher in immature animals [71].

In contrast to the enzymes of detoxification, testicular p450 activity is relatively low, about 1% of hepatic levels in the case of aryl hydrocarbon hydroxylase (AHH) activity in the rat [71]. Similarly, rat ovarian p450 and AHH is between 0.5 and 5% of liver activity [72].

Some enzymes of xenobiotic metabolism can be detected in the rodent conceptus, at preimplantation stages, and during organogenesis, particularly in the visceral yolk sac. However, activities are very low, and there has been much debate on their functional relevance. It has been suggested that these activities may be higher in primate embryos than in rodents, and this has also been an issue of discussion in relation to the extrapolation of data from animals to man, without any general consensus emerging. Late fetal development of rat and rabbit liver styrene monooxygenase, epoxide hydrolase, and glutathione-Stransferase activities have been described [70; 100; 101].

6.2 HUMAN

6.2.1 Exposure, absorption and distribution

There is a large body of data on the uptake of styrene in humans exposed experimentally and occupationally. Disposition after inhalation exposure in various conditions has been described [62; 126; 127.] A physiologically-based inhalation pharmacokinetic model has been proposed ". Absorption and excretion after skin exposure have also been studied [10; 124].

The measurement of urinary levels of styrene metabolites has been proposed, in order to generate various biological indicators of exposure [23; 90; 114; 125.] The American Conference of Governmental Industrial Hygienists (ACGIH) has published a notice of intent to establish a Biological Exposure Index (BEI) for styrene, as for some other chemicals (see 64). Personal samplers for styrene exposure have also been described *(e.g.* [16]).

6.2.2 Distribution–transplacental

It has been reported that styrene is present in human cord blood samples from the general population [19], but the data are of poor quality. Maternal and cord blood samples from 11 mother/baby pairs were analyzed by GC-MS methods. These were routine samples taken during normal deliveries. No data on maternal occupation or other potential exposures are given. Some maternal samples were taken before delivery, some immediately after. The numbers of each are not given.

It is stated that styrene was identified in maternal samples by GC-MS, but mass-ion data are not presented. It is also stated that levels of styrene were greater in those samples taken after delivery, but there are no quantitative data throughout the paper, and the numbers of samples must have been small. The authors suggest that chemicals leached from plastic infusion equipment during delivery may account for these higher levels. However, the authors also state that all women were given intravenous dextrose upon admission to the delivery unit, which was presumably prior to taking the pre-delivery maternal blood samples.

Some chemicals (benzene, carbon tetrachloride and chloroform) are stated to be present in cord blood at higher concentrations than in maternal blood. This comparison appears to refer to maternal samples taken before de-

livery, which may not be appropriate, given the finding on apparent maternal absorption of compounds during delivery. The impression given in the text is that styrene was also found in cord blood samples, but this is not explicitly stated, nor is it possible to tell with certainty from the published GC profiles. In short, this paper presents no useful information on transplacental passage of styrene in humans.

6.2.3 Metabolism—reproductive organs and conceptus

There is no information available on the styrene metabolising activity of human testes, ovary or accessory reproductive organs.

In contrast, Rane and his group at the Karolinska Institute, Sweden, have published extensively on human fetal and placental metabolism, using styrene as a model substrate [28; 81; 83; 84; 85; 86; 87; 89]. In general, enzymatic activities are low, but detectable, in fetal tissue. Enzymes of activation seem to develop later than those of detoxification. Nuclear enzymes appear to develop earlier than microsomal or cytosolic enzymes. It has been suggested that nuclear enzymes may be important in those toxicities involving reactive intermediates of very short half-life and a nuclear site of action.

6.3 ADDUCT FORMATION AND MOLECULAR MECHANISMS

The reactive metabolite styrene oxide is thought to be the ultimate mediator of most of the toxic effects of styrene. The molecular mechanisms of the reactions of styrene oxide are summarized below. Other stable metabolites may play a role in neurotoxicity as enzyme inhibitors (see 5.1.1). An effect of styrene on the cytoskeleton has also been recently suggested for neurotoxicity [65].

Both the α- and β-carbons of styrene oxide react with guanine residues through the N-7 position [18; 37; 106; 107]. This is the major adduct formed with guanosine in aqueous solution, but exocyclic N^2- and 0^6- adducts also appear [37]. N7-adducts account for 80% to 90% of the reactions with DNA *in vitro* [59; 107] Depurination and ring opening in styrene-treated DNA has been described [123]. Single strand breaks (SSB) in DNA of mice treated i.p. with styrene or styrene oxide have been reported [115]. There was a slight increase in SSB in testicular DNA, which persisted from 4 to 24 hours after injection, following 8.3 $mmol.kg^{-1}$ styrene or 5.3 $mg.\ kg^{-1}$ styrene oxide. Higher SSB levels were found in kidney, liver, lung and brain. N7- adduct ($nmol.g^{-1}$ DNA) in the testis was 0.3, compared to 8, 5, 3, and 0.6 for liver, brain, lung and spleen, following styrene oxide [18].

Styrene oxide also reacts with proteins, predominantly through cysteine [18; 35; 82]. Monitoring alkylated amino acids or nucleotides in urine or blood may provide a means of molecular dosimetry in humans exposed to styrene [18; 35].

Very little is known of the relationship between sites of alkylation and developmental or reproductive toxicities. For some direct-acting methylating and ethylating agents there is evidence that O^6-guanine, but not N7- guanine or 3-adenine, adducts correlate with the induction of teratogenicity in mice[91]. This suggests that N7- adducts may not be the teratogenic lesion for these chemicals. However, another teratogen, cyclophosphamide induces SSB and cross-links in embryonic DNA [69] and limited data suggest that its metabolite, phosphoramide mustard, forms an N7- guanine adduct [9]. Overall, the significance of DNA and protein adducts to the potential reproductive and developmental toxicities of styrene is obscure.

6.4 EVALUATION

Despite the large literature on the physiological disposition of styrene, there is very little information on reproductive organs and the conceptus. All of the animal data on transplacental transfer of styrene concern the late fetal phase of rat pregnancy. There is little detail, but it appears that fetal concentrations are less than that in maternal blood, perhaps one half as much. Fetal levels are also significantly lower than most adult organs. However, full kinetic studies have not been performed and these measured fetal levels may not be peak values, whereas adult levels probably are. There have been no studies of metabolite levels in fetal tissue. There also have been no studies of relevance to teratogenicity, *i.e.* of transfer during the organogenesis phase. Given that styrene appears to have a low developmental toxic potential, additional studies may be of academic interest only.

It appears that the reproductive organs are richer in the enzymes of styrene oxide detoxification than in those of styrene activation. However, there is very little information on styrene metabolite levels in these organs *in vivo* after styrene exposure. The relationship of adduct formation to reproductive and developmental toxicity is unknown. Since styrene does not appear to be a specific toxicant for these targets, studies of adduct formation in gonads and the conceptus may be of interest.

(Dr. Brown's review will also appear in the January 1991 issue of *Reproductive Toxicology*, vol. 5, no. 1.)

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Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis

Bruce N. Ames, Ph.D. and Lois Swirsky Gold, Ph.D.
Division of Biochemistry and Molecular Biology
University of California, Berkeley

These two distinguished researchers, in this most recent paper published in *Science*, vol. 249, point out that if current progress continues we should understand the causes of the major human cancers by the close of this decade. They anticipate that these discoveries will invalidate many of the assumptions underlying current regulatory policies, particularly the utility and meaning of routine animal cancer tests. They emphasize that too little attention has been paid to the enormous background of natural carcinogens, which have led to the development of layers of natural defenses against toxic chemicals. This means that humans are "well buffered" against toxicity at low doses from both man-made and natural chemicals.

A clarification of the mechanism of carcinogenesis is developing at a rapid rate. This new understanding undermines many assumptions of current regulatory policy toward rodent carcinogens and necessitates rethinking the utility and meaning of routine animal cancer tests. At a recent watershed meeting on carcinogenesis, much evidence was presented suggesting that mitogenesis (induced cell division) plays a dominant role in carcinogenesis[1]. The work of Cohen and Ellwein in this issue [2] is illustrative. Our own rethinking of mechanism was prompted by our findings that: (i) spontaneous DNA damage caused by endogenous oxidants is remarkably frequent [3] and (ii) in chronic testing at the maximum tolerated dose (MTD), more than half of all chemicals tested (both natural and synthetic) are carcinogens in rodents, and a high percentage of these carcinogens are not mutagens [4].

Mitogenesis increases mutagenesis. Many "promoters" of carcinogenesis have been described and have been thought to increase mitogenesis or selective growth of preneoplastic cells, or both. The concept of promotion, however, has been fuzzy compared to the clearer understanding of the role of mutagenesis in carcinogenesis. The idea that mitogenesis increases mutagenesis helps to explain promotion and other aspects of carcinogenesis [2,5].

A dividing cell is much more at risk of mutating than a quiescent cell [4]. Mutagens are often thought to be only exogenous agents, but endogenous mutagens cause massive DNA damage (by formation of oxidative and other adducts) that can be converted to stable mutations during cell division. We estimate that the DNA hits per cell per day from endogenous oxidants are normally ~10^5 in the rat and ~10^4 in the human [3]. This promutagenic damage is effectively but not perfectly repaired: for example, the normal steady-state level of 8-hydroxydeoxyguanosine (1 of about 20 known oxidative DNA adducts) in rat DNA has been measured as 1 per 130,000 bases, or about 47,000 per cell [3]. We have argued that this oxidative DNA damage is a major contributor to aging and to the degenerative diseases associated with aging, such as cancer. Thus, any agent causing chronic mitogenesis can be indirectly mutagenic (and consequently carcinogenic) because it increases the probability of converting endogenous DNA damage into mutations. Nongenotoxic agents [for example, saccharin [2]] can be carcinogens at high doses just by causing chronic mitogenesis and inflammation, and the dose response would be expected to show a threshold. Genotoxic chemicals [for example, N-2-fluorenylacetamide (2-AAF) [2]] are even more effective than nongenotoxic chemicals at causing mitogenesis at high doses (as a result of cell killing and cell replacement). Since genotoxic chemicals also act as mutagens, they can produce a multiplicative interaction not found at low doses, leading to an upward curving dose response for carcinogenicity. Furthermore, endogenous rates of DNA damage are so high that it may

be difficult for exogenous mutagens to increase them significantly at low doses that do not increase mitogenesis. Therefore, mitogenesis, which can be increased by high doses of chemicals, is indirectly mutagenic, and seems to explain much of carcinogenesis [1;4;5]. Nevertheless, the potent mutagen 2-AAF [3] induces liver tumors at moderate doses in the presence of only background rates of mitogenesis. Detailed studies of mechanism, particularly in the case of apparent exceptions, are critically important.

Causes of human cancer. Henderson and co-workers [6], and others[4], have discussed the importance of chronic mitogenesis for many, if not most, of the known causes of human cancer, for example, the importance of hormones in breast cancer, hepatitis B[7] or C viruses or alcohol in liver cancer, high salt or *Helicobacter (Campylobacter)* infection in stomach cancer, papilloma virus in cervical cancer, asbestos or tobacco smoke in lung cancer, and excess animal fat and low calcium in colon cancer. For chemical carcinogens associated with occupational cancer, worker exposure has been primarily at high, near-toxic doses that might be expected to induce mitogenesis.

Epidemiologists are frequently discovering clues about the causes of human cancer, and their hypotheses are then refined by animal and metabolic studies. During the next decade, it appears likely that this approach will lead to an understanding of the causes of the major human cancers [9]. Cancer clusters in small areas are expected to be common by chance alone, and epidemiology lacks the power to establish causality in these cases [9]. It is important to show that pollution exposure that purportedly causes a cancer cluster is significantly higher than the background of exposures to naturally occurring rodent carcinogens [4].

Causes of cancer in animal tests. Animal cancer tests are conducted at near toxic doses (the maximum tolerated dose, MTD) of the test chemical, for long periods of time, which can cause chronic mitogenesis [1]. Chronic dosing at the MTD can be thought of as a chronic wounding, which is known to be both a promoter of carcinogenesis in animals and a risk factor for cancer in humans. Thus, a high percentage of all chemicals might be expected to be carcinogenic at chronic, near toxic doses and this is exactly what is found. About half of all chemicals tested chronically at the MTD are carcinogens [4].

Synthetic chemicals account for 82% (350/427) of the chemicals adequately tested in both rats and mice [4]. Despite the fact that humans eat vastly more natural than synthetic chemicals, the world of natural chemicals has never been tested systematically. Of the natural chemicals tested, approximately half (37/77) are carcinogens, which is approximately the same as has been found for synthetic chemicals (212/350). It is unlikely that the high proportion of carcinogens in rodent studies is due simply to selection of suspicious chemical structures; most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives.

The human diet consists of thousands of natural pesticides (chemicals that plants produce to defend themselves)[4]; we calculate that 99.99% (by weight) of the pesticides in our diet are natural. Of the natural pesticides that have been tested in at least one rodent species, about half (27/52) are rodent carcinogens. These 27 occur commonly in plant foods [10]. We estimate that the average intake of these pesticides is about 1500 mg per person per day [4]. By comparison, the average intake per day of residues of 100 synthetic pesticides is 0.09 mg per person per day [4]. In addition, of the mold toxins tested at the MTD (including aflatoxin), 11 out of 16 are rodent carcinogens.

The cooking of food produces thousands of pyrolysis products, and we estimate that dietary intake of these products is roughly 2000 mg per person per day. Few of these have been tested; for example, of 826 volatile chemicals that have been identified in roasted coffee, only 21 have been tested chronically, and 16 are rodent carcinogens; caffeic acid, a non-volatile carcinogen, is also present. A cup of coffee contains at least 10 mg (40 ppm) of rodent carcinogens (mostly caffeic acid, catechol, furfural, hydrogen peroxide, and hydroquinone) [4]. Thus, very low exposures to pesticide residues or other synthetic chemicals should be compared to the enormous background of natural substances.

In the evolutionary war between plants and animals, animals have developed layers of general defenses, almost all inducible, against toxic chemicals [4]. This means that humans are well buffered against toxicity at low doses from both man-made and natural chemicals. Given the high proportion of carcinogens among those natural chemicals tested, human exposure to rodent carcinogens is far more common than generally thought; however, at the low doses of most human exposures (where cell-killing and mitogenesis do not occur), the hazards may be much lower than is commonly assumed and often will be zero [4]. Thus, without studies of the mechanism of carcinogenesis, the fact that a chemical is a carcinogen at the MTD in rodents provides no information about low-dose risk to humans.

Trade-offs. Pesticide residues (or water pollution) must be put in the context of the enormous background of natural substances, and there is no convincing evidence from either epidemiology or toxicology that they are of interest

as causes of human cancer [4; 9]. Minimizing pollution is a separate issue, and is clearly desirable for reasons other than effects on public health. Efforts to regulate synthetic pesticides or other synthetic chemicals at the parts per billion level because these chemicals are rodent carcinogens must include an understanding of the economic and health-related trade-offs. For example, synthetic pesticides have markedly lowered the cost of food from plant sources, thus encouraging increased consumption. Increased consumption of fruits and vegetables, along with decreased consumption of fat, may be the best way to lower risks of cancer and heart disease, other than giving up smoking. Also, some of the vitamins, antioxidants, and fiber found in many plant foods are anticarcinogenic.

The control of the major cancer risks that have been reliably identified should be a major focus, and attention should not be diverted from these major causes by a succession of highly publicized scares about low levels of synthetic chemicals that may be of little or no importance as causes of human disease. Moreover, we must increase research to identify more major cancer risks, and to better understand the hormonal determinants of breast cancer, the viral determinants of cervical cancer, and the dietary determinants of stomach and colon cancer. In this context, the most important contribution that animal studies can offer is insight into carcinogenesis mechanisms and into the complex natural world in which we live.

(Drs. Ames' and Gold's views appeared in the August 31, 1990 issue of *Science*, Vol. 249, pages 970-971. They are reprinted here by permission. Copyright 1990, American Association for the Advancement of Science.)

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8 Current epidemiologic data point to these risk factors for human cancer: cigarette smoking (which is responsible for 30% of cancer deaths), dietary imbalances, hormones, viruses, and occupation. "[The age-adjusted mortality rate for all cancers combined except lung cancer has been declining since 1950 for all individual age groups except 85 and above" [National Cancer Institute, 1987 *Annual Cancer Statistics Review Including Cancer Trends: 1950-1985,* NIH Publication 88-2789 (National Institutes of Health, Bethesda, MD 1988), p.II.3]. Although incidence rates for some cancers have been rising, trends in recorded incidence rates may be biased by improved registration and diagnosis. Even if particular cancers can be shown to be increasing (for example, non-Hodgkins lymphoma and melanoma) or decreasing (for example, stomach, cervical, and rectal cancer), establishing causes remains difficult because of the many changing aspects of our life-style. Life expectancy continues to increase every year.

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10 A search in foods for the presence of just these 27 natural pesticide rodent carcinogens indicates that they occur naturally in the following (those at levels over 10 ppm of a single carcinogen are listed in italics): *anise, apple,* banana, *basil,* broccoli, *Brussels sprouts, cabbage,* cantaloupe, *caraway, carrot, cauliflower, celery, cherry,* cinnamon, cloves, cocoa, *coffee (brewed), comfrey tea, dill, eggplant, endive, fennel, grapefruit juice, grape, honey,* honeydew melon, *horseradish,* kale, *lettuce, mace, mango, mushroom, mustard (brown) nutmeg, orange juice, parsley, parsnip,* peach, *pear, pepper (black),* pineapple, plum, *potato,* radish, raspberry, *rosemary, sage, sesame seeds (heated),* strawberry, *tarragon, thyme, and* turnip [4]. Particular natural pesticides that are carcinogenic in rodents can be bred out of crops if studies of mechanism indicate that they may be significant hazards to humans.

11 This work was supported by National Cancer Institute Outstanding Investigator grant CA39910, by National Institute of Environmental Health Sciences Center grant ES01869 and by DOE Contact DE-ACO3-76SF00098. We thank M. Profet, S. Liss, B. Butterworth, and R. Peto for criticisms.

Articles Featured in Previous Issues

April 1990, Vol. 1, No. 1

Styrene: Perspectives on the Carcinogen Question
by Daniel P. Boyd, Ph.D., et al., Technical Consultant; Former Director of Standards, Occupational Safety and Health Administration.

The authors review and discuss the major studies to date on the possible carcinogenicity of styrene. They begin with the work of the International Agency for Research on Cancer (IARC) which in 1987 changed its classification of styrene from "not classifiable" to "possibly carcinogenic to humans", despite the lack of any new human or animal data. They point out that several major national and international organizations have disagreed with this classification and have determined that the evidence does not support the classification or regulation of styrene as a carcinogen. They believe that significant studies currently in progress will clarify some of the issues and allow the development of improved risk estimations.

The Potential Mutagenicity of Styrene and its Metabolites
by R. Julian Preston, Ph.D., Section Head, Biology Division, Oak Ridge National Laboratory.

Dr. Preston discusses the published data on mutagenicity with respect to styrene and its metabolites, the adequacy of the data and their statistical significance. He concludes that styrene is not mutagenic in in vitro assays unless there is metabolic activation. Even when there is activation, however, styrene remains non-mutagenic or is only very weakly mutagenic. The metabolite styrene oxide is mutagenic, but the role of styrene oxide as a mutagenic intermediary in the metabolism of styrene in vivo cannot be adequately assessed from existing evidence.

The Environmental Fate of Styrene
by Martin Alexander, Ph.D., Professor, Cornell University, Department of Agronomy.

Dr. Alexander reviews the research and substantive monitoring data on the fate of styrene in water, soil and the atmosphere. He concludes that the transport of styrene in nature is "very limited" because of its volatility from soils and surface waters, its rapid destruction in air, and its biodegradation in soils and surface and groundwaters. The most probable source for any human exposure is the atmosphere, especially urban air, where values up to 6.0 ppb have occasionally been recorded. However, because styrene is highly reactive and rapidly destroyed by ozone and hydroxyl radicals, it is unlikely to be transported to any significant extent, or to be a source of styrene in waters or soils. There is little possibility of styrene occurring in drinking water or entering the food chain.

Carcinogen Classification Systems: A Time for Change
by Robert J. Moolenaar, Ph.D., Project Director, Health and Environmental Sciences, Dow Chemical Company; Chairman, Scientific Committee, American Industrial Health Council, 1982-87.

The identification of a substance as a "possible", "probable" or "reasonably anticipated to be" human carcinogen sounds many alarm bells. It raises the concerns of the public, of regulators and health officials, and of corporate executives whose companies manufacture or use the substance. It can have significant health, economic, financial and legal consequences. Yet the national and international systems used to classify carcinogenicity are confusing, contradictory, unreliable—and often arbitrary. The US Environmental Protection Agency and its Science Advisory Board have recognized the deficiencies in EPA's own classification process and are seeking ways to improve it. Dr. Moolenaar discusses the problems and recommends ways to make the classifications more accurate and more meaningful to those who depend on them.