EDITORIAL RESPONSES TO THE RINSKY ARTICLE IN THE NEW ENGLAND JOURNAL OF MEDICINE INCLUDING THE EDITORIAL BY WILLIAM F. O'KEEFE

(October 15, 1987 Vol. 317, No. 16)

PROJECT	TIMING	PROGRAM FUNCTION	COST	BUDGET SOURCE
J. Rodricks - Review of ATSDR Benzene Tox. Profile	To be completed by February 29.	Rodricks is to review and critique the epidemiology, risk assessment, and data gaps sections of the Toxicity Profile for benzene. Company members are reviewing the toxicology sections.	Up to $6,000. The original contract was for $20,000, but upon review of the ATSDR document, the level of effort was reduced.	1987 funded
J. Rodricks - Benzene stopping points effort - Phases I and II	To be completed within 2 months.	Phase I is to develop a paper outlining the problems and suitable alternatives for the use of upper bound risk estimates for setting standards under Section 112 of the Clean Air Act. Phase II is to outline the options available to EPA in making acceptable risk decisions subsequent to the Vinyl Chloride decision.	Up to $22,000 from 1987 funds provided in the contract to review the ATSDR benzene tox. profile.	1987 funds, contract amendment
J. Rodricks - Phase III	To be conducted in 1989.	This effort would develop a detailed methodology for developing acceptable risk levels under Section 112 of the Clean Air Act. Input gained from EPA's remand proceedings of the benzene standards under Section 112 will be a key portion of this effort. The objective is to help EPA evolve a workable set of alternatives for use in setting air toxic standards subsequent to the Vinyl Chloride decision.	Up to $50,000	1989 budget proposal under benzene regulatory response.
J. Sample - Exposure assessment for ambient benzene	To be completed in June 1988.	This project has three parts: (1) to provide ambient exposure data from TEAM (and other data sources) for use in developing benzene pharmacokinetic models (see Oak Ridge project below) ($15,000); (2) to develop human exposure pattern indices to address a more realistic definition of human exposures and maximally exposed individual ($5,000); and (3) to assess the sources of ambient benzene exposure ($10,000).	$30,000	1987 funded
PEI - Research on exposure/behavior patterns	To be completed in summer 1988.	This project supplements Task 2 of the exposure assessment project above by providing technical and modeling support for the development of exposure pattern indices.	$10,000	Pending HEGC approval
Carbon 14 Development Effort	To be completed in summer 1988.	This project supplements Task 3 of the exposure assessment project by providing technical and analytical support for ^{14}C analysis to differentiate fossil fuel versus non-fossil fuel sources of benzene in the human breath.	$40,000	Pending HEGC approval

long-term survival among patients with Stage III disease is 25 percent (two of eight). Among patients with Stage IV disease, 6 of 34 (18 percent) have survived overall, of the 23 patients with bone metastasis and bone marrow involvement at diagnosis — a group generally considered to have a particularly poor prognosis, 5 (22 percent) have survived. The survival of these five patients indicates that marrow "purging" may not always be needed.

There has been a price for survival — considerable treatment-related chronic toxicity. Six of the eight children have auditory injury affecting their hearing range[1] and wear hearing aids. Five patients have reduced glomerular filtration rates,[4] two of whom have chronic renal failure; one of them requires antihypertensive therapy. Apart from these handicaps, the eight children (now 6.3 to 12.4 years old; median, 8.2) are leading full and active lives, including normal school attendance.

This series is relatively small, but a comparable rate (71 percent) of response to this chemotherapeutic regimen plus surgery has been observed in a larger study by the European Neuroblastoma Study Group.[5] Previous reports state or imply that no progress has been made over the past 10 years in treating advanced neuroblastoma and that the number of survivors who are more than one year old at diagnosis is negligible.[6,7] We challenge these assertions. Our experience suggests that the median survival of patients in Stage III and IV has been extended. High-dose melphalan is probably an important component of this improved prognosis[8]; therefore, the long-term results at centers using more intensive "consolidation" regimens[8-10] will be awaited with great interest. Rather than adopt a negative attitude, those treating children with neuroblastoma should energetically search for better induction and consolidation regimens so that response rates can be improved.

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AIDS AND RENAL FAILURE

To the Editor: Rao et al. observed accelerated wasting and early death in patients with the acquired immunodeficiency syndrome (AIDS) who required maintenance hemodialysis for therapy of end-stage renal disease (April 23 issue).[1] The course appears to be more rapid than has been observed in patients with AIDS who do not have renal failure. This observation may indicate that patients with renal involvement have more extensive human immunodeficiency virus–related disease and therefore die sooner. However, it is also possible that hemodialysis accelerates the progression of AIDS. Normal immune stimulation of T helper cells may trigger replication of the AIDS virus.[2] Furthermore, patients receiving maintenance hemodialysis have an increased proportion of activated T cells in peripheral blood, as defined by expression of interleukin-2 receptors.[3] Whether such activation of T cells reflects long-term exposure to blood transfusions, infectious agents, or dialysis membranes or tubing, or is a manifestation of the uremic state itself, is unknown. Intravenous drug use, present in a high proportion of the patients studied by Rao et al., would also be associated with chronic immune stimulation. It might be interesting to see whether patients on long-term ambulatory peritoneal dialysis have increases in immune activation of T lymphocytes comparable to the increases observed in patients on maintenance hemodialysis. If increased T-cell activation is more marked in maintenance hemodialysis than in other forms of therapy of end-stage renal disease, survival of patients with this disorder and AIDS could be enhanced by an alternative therapy (such as long-term ambulatory dialysis, if feasible).

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BENZENE AND LEUKEMIA

To the Editor: In the study on benzene and leukemia by Rinsky et al. (April 23 issue),* there were but nine cases of leukemia and four cases of myeloma. These are very small numbers on which to base conclusions, especially when the overall mortality has been as expected and the mortality due to malignant neoplasms has also been as expected.

Of special interest is the authors' Table 4, which lists the cases. Some had minuscule exposure, and one wonders what other malignant disease–causing agents they may have been exposed to during their lives. Of special interest is the fact that no employee first exposed after 1954 had leukemia or myeloma, even though hiring continued for 11 years and even though 7 of the 13 cases had the onset of disease within 20 years of first exposure. (Cases were followed through 1981, so there should have been some cases.) In the study, two subjects died within 3½ years of exposure.

Looking at the year of initial exposure of the 13 cases, one can see that 9 had first exposures before the end of World War II. One wonders about the measuring instruments in that situation.

Having spent some time studying childhood malignant disease more than 25 years ago, I am very sensitive to the tendency to observe clusters of cases sometimes living within short distances of each other. Statisticians have generally attributed these to chance, and there is no reason why that may not be true in the cases in this study.

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*Rinsky RA, Smith AB, Hornung R, et al. Benzene and leukemia: an epidemiologic risk assessment. N Engl J Med 1987; 316:1044-50.

To the Editor: The paper by Rinsky et al. on workers in rubber hydrochloride plants exposed to benzene reports a cohort study, a case–control study, and a risk assessment for leukemia in relation to benzene. Table 1, which uses data from that paper, indicates a discrepancy between the cohort and case–control studies. The case–

Table 1. Deaths from Leukemia, the Standardized Mortality Ratio (SMR), and the Predicted Odds Ratio, According to Cumulative Exposure to Benzene.

Cumulative Exposure (ppm-yr)	Deaths from Leukemia	Observed SMR	Predicted Odds Ratio*
0–40	2	1.1	1.3
40–200	2	3.2	4.5
200–400	2	11.9	43.8
>400	3	66	423

*From the risk-estimation model of Rinsky et al., using the midpoint of each exposure category (480 ppm-years for highest category).

control risk assessment predicts, in the higher-exposure categories, odds ratios much larger than the standardized mortality ratios observed in the cohort. A possible explanation for the discrepancy is that the exposure of the controls was much lower than that of all cohort members without disease. In support of this, it can be inferred from the paper that the mean cumulative levels of benzene exposure of the 1156 subjects without disease and the 90 controls were about 67 and 50 parts per million (ppm)-years, respectively.

It is reasonable to ask why the case–control study was done. A case–control study within a cohort is usually done to reduce the cost of determining the exposure of all cohort members without disease. However, this reason is not applicable here, since the exposure to benzene of all cohort members was known. Rinsky et al. offer several reasons for doing the case–control study, but all the objectives could have been met effectively by a cohort analysis of all the data.

The disparity between the mean exposure level of all cohort members without disease (67 ppm-years) and that of the 90 controls (50 ppm-years) requires explanation. It is probably not the result of selection due to matching, since this should have raised the level in the comparison series. The investigators may have reviewed the information on the benzene exposures of cases and controls, with the result that the average level of the controls, but not that of the cases, was lowered. If such a review explains why the control exposure levels were lowered by 26 percent, then the validity of the cohort study is questionable. Finally, if chance explains the difference between the cohort data and the case–control risk assessment, a risk assessment based on the entire cohort would be superior. Irrespective of the explanation, it would be appropriate to evaluate the data further. Although this might involve reevaluation of the benzene exposures of all cohort members, the results should clarify the relation between benzene and leukemia.

The Occupational Safety and Health Administration (OSHA) proposes to reduce the benzene standard of a 10 ppm time-weighted average to 1 ppm. A principal basis for this is the results of the rubber hydrochloride study. However, the magnitude of the dose–response relation in this cohort is uncertain. We, in a review supported in part by Texaco, Inc., and others have done risk assessments based on this study and estimate that exposure to 300 ppm-years of benzene would cause 50 to 80 excess leukemia deaths per 1000 workers so exposed.* The risk assessment of Rinsky et al. predicts 250.

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*Austin H, Delzell E, Cole P. Benzene and leukemia: a review of the literature and a risk assessment. Am J Epidemiol (in press).

To the Editor: In his editorial on new scientific evidence and public health imperatives (April 23 issue),[1] Dr. Ashford stated that "another example is the reanalysis of the National Cancer Institute data on human exposure to formaldehyde,[2] which now indicates a clear risk of cancer to humans." The authors of the study of humans exposed to formaldehyde[2] concluded that "these data provide little evidence that mortality from cancer is associated with formaldehyde exposure at the levels experienced by workers in this study." Since substantial numbers of health workers are exposed to formaldehyde, it would be of considerable interest if Dr. Ashford could provide the evidence that indicates "a clear risk of cancer to humans."

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1. Ashford NA. New scientific evidence and public health imperatives. N Engl J Med 1987; 316:1084–5.
2. Blair A, Stewart P, O'Berg M, et al. Mortality among industrial workers exposed to formaldehyde. JNCI 1986; 76:1071–84.

To the Editor: The editorial by Ashford sounds a false alarm about the state of public health regulation today. Far from "waiting for the bodies to fall," federal agencies long ago adopted the "preventive public health policies" that Professor Ashford now pleads for. The Supreme Court's decision in the benzene case accepts this approach by authorizing OSHA to "risk error on the side of overprotection rather than underprotection."[1] The essence of the court's ruling is that this principle must be applied within realistic limits, so that instead of seeking "to eliminate completely and with absolute certainty any risk of serious harm," regulators must fairly evaluate all the available scientific data and "make a rational judgment about the relative significance of the risks" involved.[1] Federal agencies have recognized that prudence, rather than an ill-conceived rush to judgment, is crucial to sound public health regulation and have had no difficulty implementing the vital risk assessment and policy-making disciplines contemplated in the benzene decision.[2–4] In fact, OSHA has already regulated asbestos[5] and ethylene oxide[6] as carcinogens and will shortly prescribe stricter limits for formaldehyde.

The report by Rinsky et al. on a much-studied cohort of benzene workers, which Professor Ashford cites as "better science" requiring an immediate "governmental response," actually illustrates the pitfalls of reacting uncritically to a single publication. Indeed, it fails to meet the standard of scientific excellence to which he subscribes. During OSHA's public hearings on benzene last summer,[8] major shortcomings in the paper by Rinsky et al. were uncovered, including selective use of the available data on industrial hygiene, substantial underestimation of exposures, particularly during the 1940s, at the work stations where the excess cases of leukemia occurred and where workers were known to have died from the acute blood-poisoning effects of benzene[8], and failure to account for the prolonged, heavy exposures to benzene that many of the workers in this cohort experienced at work stations excluded from the analysis.[9,10]

There is nothing to be gained and much to be lost by fomenting a crisis atmosphere based on a flawed analysis that greatly exaggerates the level of risk, especially since average benzene exposures in the petroleum industry and elsewhere have for some time been reduced to levels comfortably below the 1-ppm limit OSHA has proposed.[11] Instead, Professor Ashford should recognize, as the American Petroleum Institute does, that the best way to improve health protection against the hazards we face today — occupational and otherwise — is to insist on a thorough scientific review of all the available data, focusing on the question of what exposures pose significant risks.

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7. Department of Labor. Semi-annual regulatory agenda. Fed Regist. April 27, 1987; 52:14541
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The above letters were referred to the authors of the articles in question, who offer the following replies:

To the Editor: Dr. Bader is concerned that our analysis rests on only nine deaths from leukemia and four from multiple myeloma and may therefore represent merely a description of a random "cancer cluster." That conclusion is highly unlikely, because of the close association of the cases with an experimentally proved carcinogen[1-3] and the demonstrated strong, internally consistent dose–response relation. Similar features are characteristic of series of cases of other chemically induced cancers, such as mesothelioma in asbestos workers[4] and hepatic angiosarcoma in vinyl chloride workers.[5] Dr. Bader is correct in noting that no cases of leukemia developed in our series among workers first exposed to benzene after 1954. The most likely explanation for this finding is a reduction in cumulative exposure; relatively few workers were hired after 1954, and among those workers, total lifetime exposure was kept low by decreases in production and progressive diminution in exposure standards.

Austin et al. are concerned by what they perceive as "discrepancies" between the risk estimates derived from our case–control and standardized mortality ratio analyses. Given the different nature of these two analyses, such concern is misplaced. The standardized mortality ratio study is intended simply to delineate overall patterns of mortality. It provides a poor basis for ascertainment of an exposure–response relation, because each dose category within the cohort is a distinct subset with a different duration of follow-up, a different proportion of deaths, and different age distributions. In addition, the standardized mortality ratio uses an external population for comparison — namely, the entire population of the United States. By contrast, the case–control study provides an elegant means for modeling cumulative dose while controlling for age, duration of follow-up, and other potential confounders and effect modifiers. Furthermore, it employs an internal comparison group. All that said, however, the apparent discrepancy noted by Austin et al. is largely an artifact of their manner of representing our data. They arbitrarily chose to derive risk estimates by using the midpoint of each of our exposure categories. A more appropriate procedure, given the stated highly skewed distribution of the cumulative exposures, would have been to use the person-years weighted average exposure for each category. With that approach, more highly concordant risk estimates would have been obtained.

Austin et al. are also concerned by a "disparity" between the mean level of exposure to benzene of all cohort members without disease (67 ppm-years) and that of the 90 controls (50 ppm-years). This apparent discrepancy is easily explained. The appropriate procedure for tabulating doses in a case–control incidence study requires that cumulation of the controls' exposure be truncated at the time of death of each corresponding case.[6] For all other members of the cohort, dose continues to accumulate until cessation of exposure. Austin et al. have overlooked this methodologic point, and thus the two average doses cited by them are not comparable.

We are reassured that a separate risk assessment undertaken recently by Austin et al. produced overall findings quite similar to ours.[7] Both studies demonstrate an elevated risk of leukemia with cumulative exposure to benzene, both derive similar dose–response curves, and both indicate that there is a substantial risk of leukemia at exposure levels below 10 ppm.

Mr. O'Keefe states that during public hearings on benzene held by the U.S. Department of Labor in 1986, "*major shortcomings*" were uncovered in our research. O'Keefe fails to note that with our cooperation, he and his staff scrutinized each datum in our analysis. They found only minor discrepancies among thousands of coded work histories and exposure measurements. We subsequently corrected each of these errors, and the corrections are included in our published report. Correction of those errors actually increased our estimates of leukemia risk slightly. Furthermore, the environmental measurements were reviewed by the company in whose plants the study was undertaken. The company took no exception to our characterization of exposures, and they rejected the notion that we had substantially underestimated exposure.[8]

We are pleased to note that on September 1, 1987, OSHA announced a reduction in the standard for permissible occupational exposure to benzene from 10 to 1 ppm.

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1. Snyder CA, Goldstein BD, Sellakumar AR, Bromberg I, Laskin S, Albert RE. The inhalation toxicology of benzene: incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol Appl Pharmacol 1980; 54:323-31.
2. Maltoni C, Conti B, Cotti G. Benzene: a multipotential carcinogen: results of long-term bioassays performed at the Bologna Institute of Oncology. Am J Ind Med 1983; 4:589-630.
3. NTP technical report on the toxicology and carcinogenesis studies of benzene in F344/N rats and B6C3F1 mice (gavage studies). Research Triangle Park, N.C.: National Toxicology Program, 1986 (Publication no. 86-2545).
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8. Letter from Goodyear Tire and Rubber to NIOSH, October 5, 1981. In: Occupational Safety and Health Administration. Benzene. Docket H-059. Washington, D.C.: Occupational Safety and Health Administration, Technical Data Center, 1986.

To the Editor: Despite assertions to the contrary by Mr. O'Keefe, federal (regulatory) agencies have *not* adopted preventive health policies. OSHA waited 15 years (until 1986) to regulate asbestos as a carcinogen — and then suspended the new standard for non-asbestiform fibers. This could hardly be called a preventive approach, since thousands of asbestos workers died because of the delay. OSHA also failed to establish a short-term exposure level.

Ethylene oxide was only recently regulated because OSHA was sued; OSHA has still refused to define a short-term exposure level for ethylene oxide that would be required for adequate protection. What Mr. O'Keefe would probably call an ill-conceived rush to judgment is what those concerned with public health would recommend as a prudent measure. Sufficient evidence to justify a standard is, like beauty, in the eye of the beholder.

Decisions about whether to regulate can be in error because of uncertainties about the nature and extent of the risk or about the economic and technological feasibility of regulatory controls. One type of error is committed when, because of insufficient evidence, society fails to regulate an activity that turns out to be harmful. Another type of error is committed when society regulates an activity that turns out not to be harmful and resources are needlessly expended. Aversion to making these two types of errors reflects differing values regarding the nature of the mistakes made and the extent, prevalence, or magnitude of the mistakes.

In my opinion, reliance on scientific conventions of causality for public health purposes is simply too costly in terms of human life. To dignify a decision not to regulate or a decision to wait for more evidence, under the mantle of its being scientific, is to do a disservice to both science and public policy. Furthermore, it obscures the value judgments made.

With regard to the regulation of chemicals over the past 17 years, I cannot think of a single regulated substance for which the evidence has not grown more convincing with time or for which a reversal in regulatory policy occurred. Sooner or later, the tip of the

iceberg reveals the iceberg. We are very far from ill-conceived rushes to judgment. On the contrary, the regulatory agencies have sometimes been dragged kicking and screaming into action necessary for fulfilling their statutory mandates. In the meanwhile, workers and citizens are harmed.

An example is the evolution of science regarding the carcinogenicity of formaldehyde. The National Cancer Institute (NCI) study in 1986 asserted that there was "little evidence that mortality from cancer is associated with formaldehyde exposure at levels experienced by workers in this study." In a later publication[1] the same authors concluded that

> Despite small numbers, the dose-dependent association of nasopharyngeal cancer with exposure to formaldehyde and particulates deserves further investigation through case-control studies, where the influence of formaldehyde and particulates may be evaluated with more statistical power than by standard cohort studies.

Further analysis of the NCI data by Sterling and Weinkam[2] reveals a clearly increased risk of lung cancer. At congressional hearings in 1986, the Director of the NCI, in commenting on the earlier work, testified that "in association with particulates, there is an association between formaldehyde and nasopharyngeal cancer, which clearly has to be followed up."[3]

The irony concerning the criticisms of the adequacy of the science is that the scientific evidence is essentially there. OSHA has just decided to reduce the permissible exposure level for benzene to 1 ppm. It waited far too long. Let there be a better governmental response to the new realities.

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1. Blair A, Stewart PA, Hoover RN, Fraumeni JF Jr, et al. Cancers of the nasopharynx and oropharynx and formaldehyde exposure. JNCI 1987; 78: 191-2.
2. Sterling TD, Weinkam JJ. Submitted for the record at the OSHA Hearings on Formaldehyde. Submission 182B. November 1986.
3. Formaldehyde Study. Hearing before the Subcommittee on Oversight and Investigations of the Committee on Energy and Commerce, House of Representatives, July 28, 1986: 106.

THE EC–IC BYPASS STUDY

To the Editor: The recent discussion (March 26 issue)[1-4] of the extracranial–intracranial (EC–IC) arterial bypass study is very timely. I would like to speak to one statement in the editorial: "The conclusions of the EC–IC trial are valid for the population of patients studied."[1]

The population that underwent surgery included patients who had only one episode of the transient ischemic attack. Most neurosurgeons who are experienced with the bypass procedure would not accept for surgical consideration any patient with a single transient ischemic attack. There is no discussion in the original report of the randomized study[5] about how many of the patients who underwent surgery were in this ordinarily nonoperative group. Subsequent reports by the authors of the randomized study have never clarified this issue.

Regardless of whether the randomization of the patients was proper or not, the mere fact that patients with one transient ischemic attack were included in the study negates the concept that the conclusions of the EC–IC trial are valid for the population of the patients studied.

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2. Sundt TM Jr. Was the international randomized trial of extracranial–intracranial arterial bypass representative of the population at risk? N Engl J Med 1987; 316:814-6.
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4. Barnett HJM, Sackett D, Taylor DW, et al. Are the results of the extracranial–intracranial bypass trial generalizable? N Engl J Med 1987; 316: 820-4.
5. The EC/IC Bypass Study Group. Failure of extracranial–intracranial arterial bypass to reduce the risk of ischemic stroke: results of an international randomized trial. N Engl J Med 1985; 313:1191-200.

To the Editor: The Special Reports in the *Journal* of March 26 on the validity of the international randomized trial of extracranial–intracranial arterial bypass, first published in the *Journal* in 1985,* induce some thoughts about the findings. Specifically, the implication of the findings for a better understanding of the pathogenesis of ischemic stroke is of interest.

The results are fully consistent with the hypothesis that ischemic strokes are largely embolic in origin. The relations among transient ischemic attacks, completed strokes, and atherosclerotic plaques of the internal carotid artery were well established before the study was done.

Emboli from ulcerated plaques or occlusive lesions of the internal carotid artery or middle cerebral artery would presumably lodge in a brain vessel distal to the anastomosis, whose only effect could then be to improve marginal or collateral circulation to the periphery of the territory where the infarct occurred.

Is it too simplistic to assume that in the case of the carotid lesions, a natural bypass already exists — the circle of Willis?

One of the postulates of the study was that lesions of the middle cerebral artery would be especially suitable for bypass surgery. In fact, the existence of such lesions generally indicates more severe, widespread atherosclerotic disease, with the attendant risk of stroke from embolism originating in the internal carotid artery or aorta or death from coronary heart disease.

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*The EC/IC Bypass Study Group. Failure of extracranial–intracranial arterial bypass to reduce the risk of ischemic stroke: results of an international randomized trial. N Engl J Med 1985; 313:1191-200.

To the Editor: The *Journal* is to be congratulated for its complete handling of the controversy surrounding the EC–IC operation.[1-4] However, we strongly disagree with the conclusions of both the neurosurgeons[3] and the Editor,[1] who seems to have been impressed by the neurosurgeons' arguments and to cast a shadow on the proper interpretation of such trials. We agree that generalizability is strengthened when most eligible patients are randomized, but we insist that the results should stand unless or until an even better study repudiates them. In the meantime, those who insist on using this now-discredited operation should not expect third-party reimbursement until they provide credible (i.e., well-controlled experimental) evidence that contradicts the EC–IC study.

The called-for retrospective review of the outcome in the nonrandomized patients would be as worthless as any uncontrolled historical survey. It would be impossible to sort out selection factors from treatment effects. Some patients selected for operation rather than randomization might do worse than those randomized, because the more threatened patients were treated surgically, and some might live longer because their good condition made them attractive surgical candidates. Whichever predominated, the result would be uninterpretable, as demonstrated by several published comparisons of survival of randomized and nonrandomized patients who seemed to have the same prognosis.[5-9] If surgeons have reservations about the validity of randomized controlled trials because their patients were not entered, the problem is easily solved by including all eligible patients in future studies that have the prior approval of those surgeons.

In our experience, patients readily accept randomization when their physicians are convinced of its validity. When told of the results of the trial and the lack of any evidence favoring surgery, they would prefer randomization to an operation based on surgical judgment alone. As suggested by one of us,[2] the study could be paid for with the money saved by operating on only half as many patients. In the meantime, there is no acceptable evidence that the

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Possible implications from results of animal studies in human risk estimations for benzene: nonlinear dose-response relationship due to saturation of metabolism

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Summary. To date, all risk assessment studies on benzene have been based almost exclusively on epidemiological data. We have attempted a more integrated and quantitative evaluation of carcinogenic risk for humans, trying to utilize, in addition to the epidemiological data, all data available, specifically data on metabolism, genotoxicity, and carcinogenicity in small rodents. An integrated evaluation of the globality of the available data seems to suggest a progressive saturation of metabolic capacity both for man and rodents between 10 and 100 ppm. The most susceptible target cells seem to be different in humans (predominant induction of myelogenous leukemia) and small rodents (induction of a wide variety of tumors). Nevertheless, both epidemiological and experimental carcinogenicity data tend to indicate a flattening of the response for the highest dosages, again suggesting a general saturation of mechanisms of metabolic activation, extended to different target tissues. From a quantitative point of view, the data suggest a carcinogenic potency at 10 ppm two to three times higher than that computable by a linear extrapolation from data in the 100 ppm range. These observations are in accord with the recent proposal of the European Economic Community of reducing benzene time-weighted average occupational levels from 10 to 5 ppm.

Key words: Benzene – Risk estimation – Carcinogenicity – Genotoxicity – Metabolism saturation – Dose-response relationship

Introduction

Benzene is probably the chemical with the largest production and utilization in the world (IARC 1982a). As a consequence, both occupational exposure and environmental contamination occur to a rather significant extent. Benzene has been recognized for many years as a carcinogen in humans with the induction of acute myelogenous leukemia (IARC 1982a). The most common regulatory standard for occupational exposure in industrialized countries is a limit of 10 ppm (time-weighted average TWA) (IARC 1982a). For the corresponding risk assessment often only epidemiological data have been considered (IARC 1982a). Correlations with carcinogenicity data in rodents have less frequently been established (Lee et al. 1983).

In this paper, we have tried to enrich current information by compiling the data related to genotoxicity versus nongenotoxicity in the mechanism of action of benzene, by comparing metabolism in humans and rodents, and by pharmacokinetic considerations of a possible saturation of benzene metabolism and genotoxicity at higher exposure levels.

Epidemiological data

In the annex to the IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol 29 (IARC 1982b), a working group attempted a quantitative risk estimation for benzene, based entirely on human epidemiological data. Minimum estimates of 14–170 excess leukemia deaths per 1000 workers exposed over a working lifetime were calculated for an exposure level of 100 ppm. Their computations were based on three epidemiological studies (Vigliani 1976; Ott et al. 1978; Rinsky et al. 1981) for which ranges of excess cases and exposure levels are shown in Fig. 1. Using the words of the authors (IARC 1982b): "The working group found that quantitative estimation was more feasible than seemed initially to be the case. The group was impressed that rather large amounts of quantitative information could be extracted from the published epidemiological data and that reasonable risk estimations could then be based on that information."

The IARC estimate is in rather good agreement with the estimate of Goldstein (1985). The lifetime risk per parts per million computed by this author was apparently about 10 times higher than the lifetime risk computed by the IARC Group. The difference is only apparent and due to the fact that in the Goldstein computations a continuous life exposure was considered, whereas in the computa-

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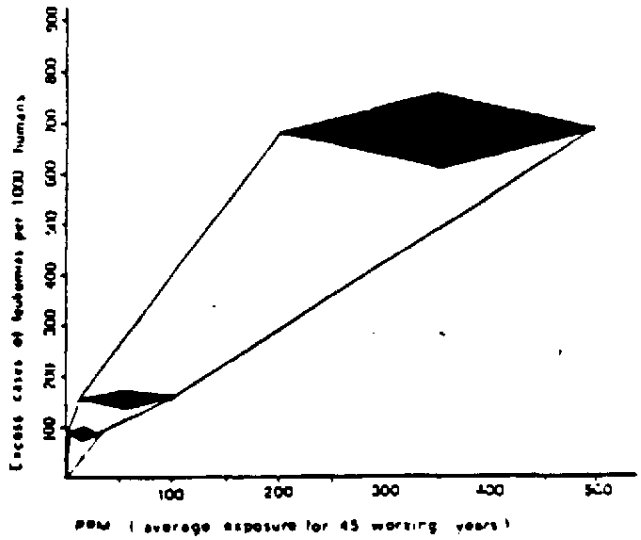

Fig. 1. Leukemia incidence in workers exposed to benzene (computations made by IARC Working Group, IARC 1982a). Ranges of benzene concentration include variations in exposure levels and uncertainty in analytical determinations performed by "old" methods which had a precision of ± 50% (Runsky et al. 1981)

tions of the IARC Group the exposure was referred to 40 h week for 45 years. When the computations of Goldstein are corrected according to the standard of exposure utilized by the IARC Group, the excess cases of leukemia deaths expected for 100 ppm become 332/1000 workers, in good agreement with the value deduced from Fig. 1. In the Goldstein computations, two out of three epidemiological studies were the same as those utilized by the IARC Group.

Carcinogenicity in animals

To our knowledge, the first suggestion that benzene could be carcinogenic in small rodents came from work published in 1979 by Maltoni and Scarnato. The induction of Zymbal gland carcinoma in female rats was statistically significant ($P = 0.0031$) at 250 mg/kg (administered by gavage once daily, 4–5 days each week, for 52 weeks).

A suggestion that benzene could be carcinogenic in small rodents upon inhalation came from a work published in 1980 by Snyder et al. Benzene was given to C57BL/6J mice at a concentration of 300 ppm, 6 h/day, 5 days/week, for life. Eight hematopoietic tumors were found in 40 treated animals, whereas only 2 were found in 40 controls. The difference was suggestive but only borderline from a statistical point of view ($P = 0.044$, one-tailed, according to the Fisher exact test).

After the publication in 1982 of the work of the IARC group on quantitative cancer risk estimation based only on epidemiological data (IARC 1982b), the carcinogenicity of benzene in small rodents was better defined und confirmed (NTP 1984; Cronkite et al. 1985; Maltoni et al. 1985). An overall picture of the results that we have considered relevant is presented in Table 1. Differences in strain susceptibility and in route of administration can be observed: Sprague-Dawley rats are less susceptible than Fischer 344 rats to benzene administered by gavage. A quantitative comparison of the carcinogenicity results from oral with inhalation exposure could not be made because we could not find enough data on serum levels of benzene at different concentrations and times after beginning of treatment.

If we confine ourselves to the high dose inhalation experiments of Maltoni et al. (1985), we can compute a tumor incidence over controls (animals with at least one tumor) of 0.27 for an average continuous concentration (on the basis of 8 h/day, 5 days a week) of 243 ppm for 2 years (a different value (TWA = 284 ppm) is reported in the legend of Table 1, but this value refers to a daily exposure of 4 h for 7 weeks plus 7 h for 97 weeks), equivalent approximately to an incidence over controls of 111/1000 animals for 100 ppm for rat lifespan. If we consider the inhalation experiment with mice of Snyder et al. (1980), we can compute a tumor incidence over controls of 0.15 for an average continuous concentration of 225 ppm for 2 years, equivalent approximately to an incidence over controls of 67/1000 animals for 100 ppm for mouse lifespan. Considering carcinogenic potencies can span a range of more than 10^7 times (Parodi et al. 1982; Peto et al. 1984), a difference by a factor

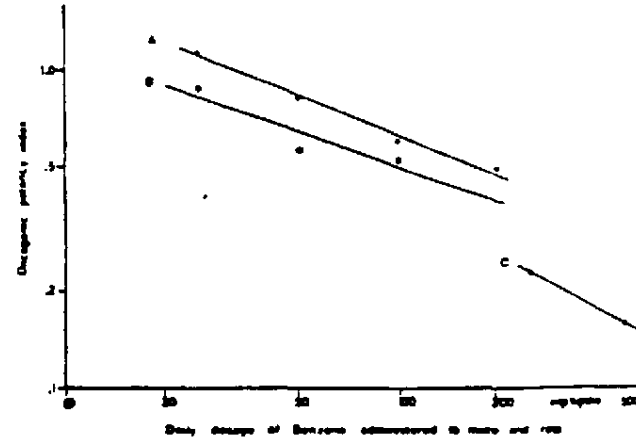

Fig. 2. Oncogenic potency index (OPI) as a function of dosage of benzene in mice and rats. $OPI = -\ln(1-I)/Dt$, where I is the excess incidence over controls (proportion of animals with at least one malignant tumor for all tumors), t is the length of observation (time unit = 2 years) and D is the dosage in millimoles per kilogram per day equivalent to the total dose divided by a 2-year exposure (Parodi et al. 1982). Only dosages which produced statistically significant effects ($P < 0.05$) were considered. These data are not directly comparable with those of Table 1 where malignant tumor incidences of different target organs are reported. A = F344 rats, gavage (NTP 1984); B = B6C3F1 mice, gavage (NTP 1984); C = S-D rats, gavage (Maltoni et al. 1985)

Table 1. Malignant tumors observed in long-term assays of benzene carcinogenicity in rodents

Species	Route of administration									
	Inhalation					Gavage				
	Main target organs	Tumor incidence over controls	TWA chronic dose (ppm)	Response	Strain	Main target organs[a]	Tumor incidence over controls	Chronic dose (mg/kg per day)	Response	Strain
Rat	Zymbal glands	0.093	284	(+ +)	S-D[b]	Zymbal glands	0.22	100	(+ +)	F344[c]
	Oral cavity	0.079[d]	284	(+ +)	S-D[b]	Zymbal glands	0.42	500	(+ −)	S-D[b]
	Liver (hepatomas)	0.108[d]	284	(+ +)	S-D[b]	Oral cavity	0.19	50	(+ +)	F344[c]
	Breast	0.084[d]	284	(+)	S-D[b]	Oral cavity	0.52	500	(+ +)	S-D[b]
	Nasal cavity	0.021	284	(+)	S-D[b]	Liver (angiosarcoma)	0.063	500	(+)	S-D[b]
						Skin	0.23[e]	500	(+)	S-D[b]
						Skin	0.16[e]	200	(+)	F344[c]
						Nasal cavity	0.05	500	(+)	S-D[b]
						Forestomach (in situ ca.)	0.15[d]	500	(+)	S-D[b]
Mouse	Hematopoietic system[f]	0.15[e]	300	(±)	C57BL.6[g]	Zymbal glands	0.24	100	(+ +)	B6C3F1[c]
	Hematopoietic system[f]	0.145[e]	300	(+)	C57BL.6[h]	Liver (adenomas + carcinomas)	0.14[e]	100	(+ +)	B6C3F1[c]
	Ovary	0.09[d]	300	(+)	C57BL.6[h]	Hematopoietic system[f]	0.16	25	(+)	B6C3F1[c]
	Zymbal glands	0.17[d]	300	(+)	C57BL.6[h]	Breast	0.275[d]	500	(+)	Swiss[i]
						Lung (adenomas)	0.22	500	(+)	Swiss[h]

[a] Tumor incidence in treated groups was higher than that of controls from a statistical point of view ($P < 0.05$). Only the most effective dosage was reported

[b] Maltoni et al. (1985). In inhalation studies, S-D rats were exposed to benzene pre- and postnatally (4–7 h/day, 5 days/week for 104 weeks (TWA = 284 ppm) or 15 weeks (TWA = 200 ppm)). Experiments with Wistar rats and Swiss mice are ongoing (100-week study). Tumor incidences from this work have been calculated by referring the number of tumor-bearing animals to the correct number (i.e., the number of survivors at the time that the first tumor appeared, regardless of the organ considered)

[c] NTP study (1984). Malignant tumors of lung, breast, and preputial glands were also induced

[d] Only in females

[e] Only in males

[f] Malignant lymphomas

[g] Snyder et al. (1980). Mice were exposed for 6 h/day, 5 days/week for life. A single dose was tested

[h] Cronkite et al. (1985). Subchronic exposure (16 weeks) and 99-week observance. A single dose was tested

TWA = time-weighted average. F344 = Fischer 344 rats. S-D = Sprague-Dawley rats. (+ +) = dose-related tumor induction. (+) = dose-unrelated tumor induction. (±) = borderline evidence of tumor induction ($0.1 > P > 0.05$)

of 2 to 5 between small rodents and humans is remarkably small. Obviously, in making this statement we imply that the ratio of tumor latency to lifespan is essentially the same for both humans and small rodents. Most experimental evidence seems to favor this broad assumption (behind this apparently simple hypothesis very complex problems are hidden when considering carcinogenesis as a multistep process. Mutation-like events seem to have a similar frequency in human and rodent cells, but perhaps the number of steps is different in the two species, in order to adjust latency to the respective lifespan). The dosage extrapolations at low levels of risk, performed by the USA Environmental Protection Agency (Lee et al. 1983; Goldstein 1985), showed a similar picture of concordance between human and animal data.

From the point of view of overall tumor frequency small rodents and humans showed very similar sensitivity. Considering target organ, only few leukemias (usually not of the myeloid type) were induced in rodents. Zymbal glands and oral cavity were the primary targets in rodents, but neoplastic effects were also observed in liver and breast, and occasionally in skin, nasal cavity, lung, forestomach, adrenals, ovary, Harderian and preputial glands (Table 1). We are aware that the application of animal data (as proposed here) to humans would be safer if not only a quantitative but also a qualitative similarity of tumor incidences had been shown in the two systems.

Some indication of a possible dose-response relationship is offered by the experiments when benzene was given by gavage. In Fig. 2 the oncogenic potency

Table 2. Short-term assays of potential carcinogenicity of benzene: evaluation of most genotoxicity data from literature. Figures indicate the number of short-term assays which were either positive (+) or negative (−). Unless otherwise indicated, data are taken from IARC (1982a, c), ECETOC (1984) and Dean (1985) reviews. Data lacking in experimental details, equivocal for the authors or for IARC working group, or successively changed by the same authors after having modified experimental design, have not been considered

Endpoint	DNA damage				Mutation				Chromosome anomalies				Other			
System	(+)			(−)	(+)			(−)	(+)			(−)	(+)			(−)
Prokaryotes	0	pol A	2		0	ST	1[a]									
					0	ST, BS	7									
					0	ST	5[b]									
Fungi green plants					0	SC	1		3[b]	A	1[b]					
					5[b]	GM, GC, CO	16[b]									
					2	TRD	0									
Insects					0	RM	1									
					1[b]	RM	2[b]									
Amphibians									1[c]	CA	0					
Mammalian cells (in vitro)	1[d]	MtMBI	0		0	L5178Y	1		1	A	0		0	MC	3[b]	
	1[d]	MMBI	0		5[b]	L5178Y,V79, CHO, HL	9[b]		1[c]	CA	0		3[b]	TR	5[b]	
	1[b]	AE	2[b]						7	CA	1					
	2[b]	UDS	4[b]						3[b]	CA	4[b]					
									2[b]	A/P	3[b]					
									2	SCE	1					
									0	SCE	6[b]					
									0	MN	1[b]					
Mammals (in vivo)	1[b]		1[f]		0		1[b]		13	CA	1		0	DL	1	
	2[b]		0						1[f]	CA	0					
									2	SCE	0					
									11	MN	0					
									1[f]	MN	0					
Humans (in vivo)									19	CA	2					
									4[b]	CA	0					
									0	SCE	2					
Class positivity:	8/17 = 47.1%				14/58 = 24.1%				71/93 = 76.3%				3/12 = 25.0%			
Overall activity (positive tests/total tests):	96/180 = 53.3%															

[a] From Lee et al. (1983)
[b] From Table 1 of ICPS collaborative study of Ashby et al. (1985)
[c] From Kalf et al. (1985)
[d] From Arfellini et al. (1985)
[e] From Parodi et al. (1983) alkaline elution
[f] From Parodi et al. (1983) viscosimetric unwinding
[g] From Lutz and Schlatter (1977b) and Arfellini et al. (1985) DNA adducts
[h] Point mutation in rat chromosomes
[i] From Gad-el-Karim et al. (1984)
[j] From Choy et al. (1985)

CHO = Chinese hamster ovary. ST = *Salmonella typhimurium*/microsome. BS = *Bacillus subtilis*. SC = *Saccharomyces cerevisiae*. GM = gene mutation. GC = gene conversion. CO = crossing-over. A = aneuploidy. RM = recombination and mutation. CA = chromosome aberration. MtMBI = mitochondria-mediated binding to mitochondrial DNA. MMBI = microsome-mediated binding to calf thymus DNA. AE = alkaline elution. UDS = unscheduled DNA synthesis. HL = human lymphocytes. P = polyploidy. SCE = sister chromatid exchange. MN = micronucleus test. MC = metabolic cooperation. TR = cell transformation. DL = dominant lethal test (rat). pol A = DNA polymerase-deficient and proficient *Escherichia coli*. TRD = Tradescantia assay

index (OPI) computed according to Parodi et al. (1982) for increasing benzene dosages is reported, and suggests a higher relative sensitivity to the carcinogenic effect of benzene at lower dosage. Carcinogenicity data in rodents for vinyl chloride given by inhalation also showed typical flattening of the response at the highest dosages (Maltoni 1975). However, in the case of benzene, dose-response experiments with inhalation exposure are not available. A quantitative correlation between human and animal data was reported by Rall (1977) for chlornaphazine, benzidine, diethylstilbestrol, and tobacco smoke.

Short-term tests

The qualitative results obtained with short-term assays are illustrated in Table 2. As is typical of a compound examined in many different short-term tests and in many laboratories, there are a mixture of positive and negative results. In order to express a global opinion on the data, we have followed the approach suggested by Ashby et al. (1985). These authors have examined the results obtained with short-term tests, performed in 66 different laboratories, on 10 different chemicals. Clearly genotoxic and carcinogenic compounds like acrylonitrile and toluidine were positive in 40%–60% of the tests. Presumed noncarcinogens and/or nongenotoxic compounds like benzoin and caprolactam were positive in about 20% of the tests. The authors suggested that potent carcinogens with a genotoxic mechanism would be positive in ~80% of the tests. Noncarcinogens could be positive in ~20% of the cases. Weak carcinogens should produce an intermediate number of positive responses.

We examined all the data that could be collected on benzene without any selection or exclusion of data. Benzene appeared positive in 53% of the cases (Table 2). In the perspective of the above considerations, the results are definitely more typical of a genotoxic carcinogen than of a noncarcinogen or nongenotoxic agent. However, looking at the results in more detail, the percent of positivity of DNA damage data was 47%, whereas for point mutations and chromosomal anomalies the values were 24% and 76%, respectively (Table 2). It seems reasonable that there might be something peculiar in the DNA damage induced by benzene, in the sense that the damage seemed clearly more clastogenic in vivo and in vitro than mutagenic. We have examined the publications available on chromosomal damage induced by benzene in rodents in terms of dose-response relationship after treatment in vivo (Erexson et al. 1984; Gad-el-Karim et al. 1984; Styles and Richardson 1984; Choy et al. 1985). The results obtained are shown in Table 3. The potency of the response (effect/dosage) clearly decreased from low to medium to high dosage. In the experiments where benzene was given by inhalation (Erexson et al. 1984; Styles and Richardson 1984) the net effect was only doubled when the exposure in-

Table 3. Dose-response relationship for the clastogenic activity of benzene in rodents

Species	Route of administration	Endpoint and dose unit	Effect[a] dosage ratio at different dosage		
			Low	Medium	High
Male Swiss mice	Oral[b]	Micronuclei 1000 PCE mg/kg	$\frac{4-2.4}{8}[c] = 0.19$	$\frac{6-2.4}{88} = 0.04$	$\frac{28-2.4}{880} = 0.03$
	Oral[b]	Chromosome aberrations per metaphase, mg/kg	$\frac{0.06-0.03}{8.8}[c] = 0.0034$	$\frac{0.16-0.03}{88} = 0.0015$	$\frac{1.1-0.03}{880} = 0.0012$
Male B6C3F1 mice	Oral[d]	Micronuclei 1000 NCE mg/kg	$\frac{2.5-1.2}{25}[e] = 0.052$	$\frac{5.8-1.2}{100} = 0.046$	$\frac{9.3-1.2}{600} = 0.013$
Male Wistar rat[e]	Inhalation[f]	% cells with chromosome anomalies, ppm	$\frac{3.03-1.54}{10}[g] = 0.15$	$\frac{4.45-1.54}{100} = 0.030$	$\frac{7.81-1.54}{1000} = 0.0063$
Male DBA/2 mice	Inhalation[h]	SCE metaphase ppm	$\frac{7.6-5.9}{10}[i] = 0.17$	$\frac{9.5-5.9}{100} = 0.036$	$\frac{13.8-5.9}{1000} = 0.0079$
	Inhalation[h]	Micronuclei 1000 PCE ppm	$\frac{0.9-0.21}{10}[i] = 0.069$	$\frac{2.03-0.21}{100} = 0.018$	$\frac{2.81-0.21}{1000} = 0.0026$

[a] Treated values minus control values
[b] Two doses were administered 24 h apart and animals were sacrificed 30 h after the first dose (Gad-el-Karim et al. 1984)
[c] Corresponding to 3.76 ppm according to EPA computations (Lee et al. 1983), assuming a respiratory absorption coefficient of 0.5 and a ventilation capacity of 0.03 l/min for mouse (Gold et al. 1984)
[d] Subchronic treatment within NTP study (1984). Micronuclei were measured after exposure for 5 days/week for 4 months (Choy et al. 1985)
[e] Corresponding to 10.7 ppm according to EPA computations (Lee et al. 1983), assuming a respiratory absorption coefficient of 0.5 and a ventilation capacity of 0.03 l/min for mouse (Gold et al. 1984)
[f] Exposure of 6 h (Styles and Richardson 1984)
[g] Corresponding to 7.1 mg/kg according to EPA computations (Lee et al. 1983), assuming a respiratory absorption coefficient of 0.5 and a ventilation capacity of 0.1 l/min for rat (Gold et al. 1984)
[h] Exposure of 6 h (Erexson et al. 1984)
[i] Corresponding to 23.4 mg/kg according to EPA computations (Lee et al. 1983), assuming a respiratory absorption coefficient of 0.5 and a ventilation capacity of 0.03 l/min for mouse (Gold et al. 1984)
PCE = polychromatic erythrocytes. NCE = normochromatic erythrocytes. SCE = sister chromatid exchanges

Table 4. Rank classification of potency of oncogenicity (OPI) and of DNA damaging activity (CBI) for 36 carcinogens tested in rat and mouse[a] and for benzene

Rank	Log_{10} OPI	Log_{10} CBI	Rank	Log_{10} OPI	Log_{10} CBI
1	−1.42 Anil	−0.35 Eth	19	2.66 CP	2.06 NHMI
2	−0.96 AAB	−0.30 DES	20	2.72 MDAB	2.07 NPI
3	−0.75 CCl_4	0.21 2-NA	21	2.73 ENU	2.14 DEN
4	−0.56 MMS	0.38 AAB	22	2.96 BP	2.16 AAT
5	0.00 $CHCl_3$	0.45 MCA	23	2.97 DEN	2.25 NPY
6	0.34 2-NA	0.57 Anil	24	3.01 AAF	2.26 Benzid
7	1.26 NPY	0.83 DAB	25	3.05 HAAF	2.26 EDB
8	1.40 Eth	0.86 $CHCl_3$	26	3.06 EC	2.29 ENU
9	1.57 EDB	1.18 BP	27	3.07 Benzid	2.59 HAAF
10	1.61 DAB	1.35 DMBA	28	3.13 DMN	2.60 MMS
11	1.65 DMH	1.41 CCl_4	29	3.43 MNU	2.71 MNU
12	1.79 NPI	1.61 EC	30	3.52 DES	2.75 AFB2
13	2.12 NM	1.64 NM	31	3.60 NMust	2.83 AFG1
14	2.14 AAT	1.79 CP	32	3.73 NHMI	3.00 MNNG
15	2.23 MNNG	1.79 EMS	33	3.73 MAMA	3.40 DMH
16	2.33 MCA	1.82 MDAB	34	3.95 AFG1	3.47 DMN
17	2.35 AFB2	1.92 NMust	35	4.36 DMBA	3.64 MAMA
18	2.50 EMS	2.03 AAF	36	4.41 AFB1	4.09 AFB1

Benzene: OPI values = 0.82[b], 0.23[c], 0.38[d], 0.086[e], 0.056[f]. Log_{10} OPI = −0.086[b], −0.638[c], −0.056[d], −1.09[e], −1.25[f]
CBI values = 7[g], 1.7[h], 15[i]. Log_{10} CBI = 0.845[g], 0.23[h], 1.176[i]

[a] Data from Parodi et al. (1982)
[b] F-344 rats, gavage (NTP study 1984)
[c] S-D rats, gavage (Maltoni et al. 1985)
[d] B6C3F1 mice, gavage (NTP study 1984)
[e] Swiss mice, gavage (Maltoni et al. 1985)
[f] Wistar rats, gavage (Maltoni et al. 1985)
[g] Wistar rats, i.p. (Arfellini et al. 1985)
[h] SIV-50 rats, inhalation (Lutz and Schlatter 1977b). Exposure in a closed inhalation chamber: the amount of benzene administered was known
[i] BALB/c mice, i.p. (Arfellini et al. 1985)
Arrows indicate the rank positions of log_{10} OPI and of log_{10} CBI of benzene
Anil = aniline. AAB = 4-aminoazobenzene. CCl_4 = carbon tetrachloride. MMS = methyl methane sulfonate. $CHCl_3$ = chloroform. 2-NA = 2-naphthylamine. NPY = N-nitrosopyrrolidine. Eth = ethionine. EDB = ethylene dibromide. DAB = N,N-dimethyl-4-aminoazobenzene. DMH = 1,2-dimethylhydrazine. NPI = N-nitrosopiperidine. NM = N-nitrosomorpholine. AAT = o-aminoazotoluene. MNNG = N-methyl-N'-nitro-N-nitrosoguanidine. MCA = 3-methylcholanthrene. AFB2 = aflatoxin B_2. EMS = ethyl methane sulfonate. CP = cyclophosphamide. MDAB = 3'-methyl-4-dimethylaminoazobenzene. ENU = N-nitrosoethylurea. BP = benzo(a)pyrene. DEN = diethylnitrosamine. AAF = 2-acetylaminofluorene. HAAF = N-hydroxy-2-acetylaminofluorene. EC = ethyl carbamate. Benzid = benzidine. DMN = dimethylnitrosamine. MNU = N-nitrosomethylurea. DES = diethylstilbestrol. NMust = nitrogen mustard. NHMI = nitrosohexamethyleneimine. MAMA = methylazoxymethanol acetate. AFG1 = aflatoxin G_1. DMBA = 7,12-dimethylbenz(a)anthracene. AFB1 = aflatoxin B_1

creased 10-fold from 10 to 100 ppm. In their work on micronuclei induction in polychromatic erythrocytes of CD-1 mice Hite et al. (1980) reported very large fluctuations in each time group after oral treatment, but the average trend was flattening of the response above 110 mg/kg, corresponding to approximately 47 ppm according to EPA computations (Lee et al. 1983) (see also legend of Table 3).

Carcinogenic potency of mutagens in rodents seems to correlate semiquantitatively with adduct formation in liver DNA. In 1979 Lutz proposed an index of potency, the covalent binding index (CBI) for DNA adducts. In 1982, Parodi et al. discussed a procedure for the transformation of carcinogenicity data in terms of potency coefficient, OPI, which is, as a first approximation, the reciprocal of the TD_{50} index of Peto et al. (1984). From a database of carcinogenic potency (OPI) and liver DNA adducts in vivo (CBI) that we had published previously (Parodi et al. 1982) we were able to find a group of 36 chemicals for which both OPI and CBI data were available. These data, in ascending rank of potency are presented in Table 4. Potency data concerning benzene were inserted at the appropriate places, between the 1st and 5th position for OPI values and between the 3rd and 9th position for CBI values. For both OPI and CBI values benzene ranked amongst the weakest chemicals on the scales of potency. The data seem to suggest not only a qualita-

tive correlation between carcinogenicity and adduct formation but also some degree of quantitative correlation.

Kinetics and metabolism

In humans, following benzene exposure at relatively low concentrations (amount of absorbed benzene ~4–10 mg/kg) (Teisinger et al. 1952; Nomiyama and Nomiyama 1974a, b), about $^1/_3$ is expired unchanged in the air and $^2/_3$ is excreted as metabolites in the urine within 24–48 h. Approximately 87% is excreted as conjugates of phenol, 9% as conjugates of catechol, and 3% as conjugates of hydroquinone. In Swiss mice injected s.c. with 440 or 880 mg/kg benzene, approximately $^2/_3$ or $^3/_4$, respectively, is excreted unchanged in the air, and $^1/_3$ or $^1/_4$, respectively, is excreted as metabolites in the urine within 24 h (Andrews et al. 1977). Other studies on the urinary metabolites in mice (Longacre et al. 1981), have indicated that 81% is represented by phenol, 15% by catechol, and 4% by hydroquinone. Trace amounts of trans-trans muconic acid and of 1,2,4-trihydroxybenzene have also been detected. Benzoquinones are, possibly, the electrophilic reacting intermediates; involvement of free radical mechanisms in phenol production has also been postulated (Johansson and Ingelman-Sundberg 1983). The same metabolites and similar ratios have been found in the urine of many other mammals (IARC 1982a), which suggests that the major pathways of benzene metabolism are similar in all mammalian species tested so far.

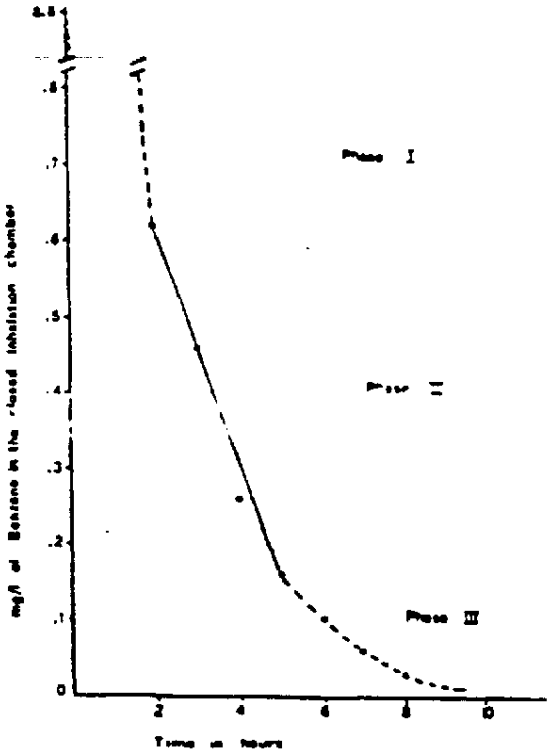

Fig. 3. Benzene concentration in the closed inhalation chamber as a function of time after injection of benzene into the chamber. In phase I, the major phenomenon is uptake and distribution of benzene in the rat. In phase II, benzene concentration decreases, essentially by zero order kinetics. In phase III, a shift to 1st order kinetics occurs

In view of these considerations it seems acceptable to utilize the information obtained from experimental data (both short-term tests, metabolism, and pharmacokinetics) for an investigation of the relationship between dose and effects. In order to investigate the kinetics of uptake and metabolism of benzene by inhalation, adult male SIV-50 rats (270–340 g) were exposed to benzene evaporated in a closed inhalation system (a 2 l desiccator) which has been described in detail elsewhere (Lutz and Schlatter 1977a). In this system, expired carbon dioxide was adsorbed on soda lime and replaced by pure oxygen to keep the total pressure at a constant level. A typical time-course of benzene concentration in the desiccator is shown in Fig. 3; similar patterns were obtained for the experiments reported in Fig. 4. During the first 2 h, the benzene concentration in the air rapidly decreased until an equilibrium was reached between absorption and exhalation. Within 2 h, about 90% of the total amount of benzene had been taken up by the rat, about 10% was in the air. In a second phase, the level of benzene in the chamber decreased slowly, always in equilibrium with the internal level of benzene so that the rate of the metabolic turnover could be estimated from the decreased benzene concentration in the air.

Using different doses of benzene, the phase II data were analyzed for the period of time required to halve the benzene concentration determined at 2 h. The results are shown in Fig. 4. In the range 200–1200 ppm (0.6–3.8 mg l), the "halflife" of benzene was not a constant value but seemed to increase linearly with benzene concentration in the air. This suggests a zero order reaction, as if the overall metabolic system was saturated and working at a maximum constant speed. A regular feature of the experiments reported in Fig. 4 was the presence of a phase III, as reported in the example of Fig. 3: starting from about 50–30 ppm and below, the system was no longer saturated and first order decay of benzene levels was regularly observed.

Other findings seemed to confirm the observation that the rate of benzene metabolism is progressively saturated and flattened at concentrations above 30 ppm. The results of Andrews et al. (1977) seemed to indicate the same, when mice (30 g) were given ^{3}H-benzene s.c., one group receiving 440 mg kg and another group receiving 880 mg kg. The metabolites in

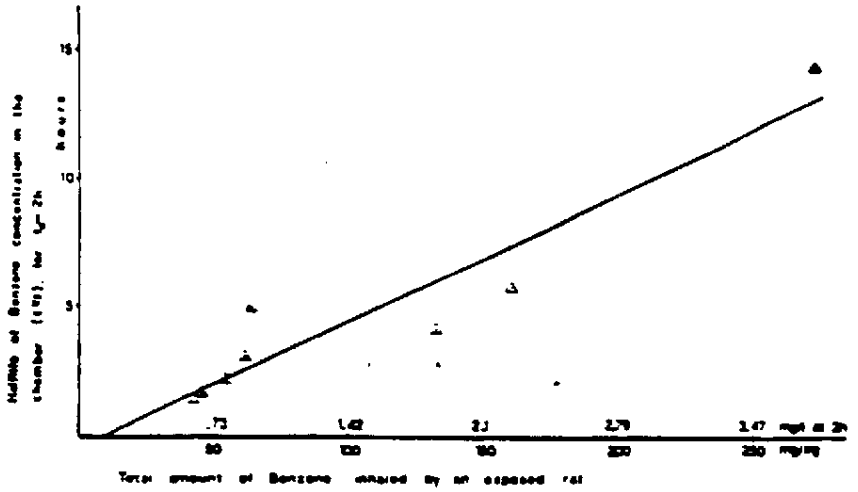

Fig. 4. Reciprocal rate of benzene consumption in rats exposed to increasing concentrations of benzene (phase II of Fig. 3) in a closed inhalation chamber (Lutz and Schlatter 1977a). On the abscissa, the total amount of benzene applied to the chamber and expressed per kilogram body weight is given along with the corresponding concentration in the chamber after 2 h. This second scale was obtained from a linear regression ($y = 0.049 + 0.014x$, $r = 0.954$, $n = 8$) between benzene concentration observed in single experiments and total amount of benzene applied

urine were measured over a 24-h period. The micromole equivalents of benzene found as metabolites in the urine were similar (60.1 and 72.6), irrespective of doubling the dose. Again, metabolic processes were saturated. Furthermore, rats inhaling benzene concentrations of 125, 250, 625, or 1250 ppm for 6 h excreted similar amounts of phenol in urine: hence benzene metabolism was already capacity-limited at 125 ppm (Gut and Frantik 1980).

Although no appropriate metabolism experiments are available in humans, the epidemiological data on the tumor incidence seem to suggest a flattening of the dose-response curve, for concentrations above 50 ppm (Fig. 1).

Conclusions

The quantitative concordance between experimental data and epidemiological data seems to allow some degree of utilization of experimental results in human risk estimation. A few years ago, in response to the publication of the IARC report on benzene (IARC 1982b) it was hotly debated (Sun 1982; Tomatis 1982) whether a linear extrapolation from 170 excess leukemia deaths (for a working lifetime exposure to 100 ppm) to 17 excess deaths (3 times background risk) for a 10 ppm exposure could be defensible.

We have presented evidence that nonlinear dose-response relationships suggested by the data on tumor induction in animals and man and on chromosomal damage in rodents might be explained by the metabolism of benzene which seems to become saturated in the dose range analyzed. A similar situation has been described previously for vinyl chloride where it was shown that metabolism (Gehring et al. 1978), macromolecular binding (Watanabe et al. 1978), and tumor induction (Maltoni and Lefemine 1975) all become saturated at inhalation exposure levels between 500 and 2500 ppm. The threshold with benzene appears to be at a lower concentration in the rat (30–50 ppm) and it remains to be elucidated whether saturation of benzene metabolism in humans occurs in the same exposure range. Since this cannot be investigated in vivo, alternative methods might be required. One possibility would be the use of human tissue samples for the study of the maximum metabolic capacity in liver and bone marrow.

Because of the flattening of the curves at high exposure levels, probably due to saturation of the activation of benzene to reactive metabolites, the data obtained at the lowest exposure should be considered the most relevant as a basis for an extrapolation. Data obtained from dose levels above saturation will be of less value for defining the shape of the dose-response curves in the lower range. It is inevitable that a 10 ppm exposure will result in a higher risk than if the high dose data are given the same weight, regardless of the model chosen. Our utilization of the available information tends to suggest that going from 100 ppm to 10 ppm for a 45-year exposure the risk could be two to three times higher than that expected from a linear extrapolation. Usually benzene workers are exposed for an average of about 5–10 years and not for 45 years; therefore, we are dealing with a small increment of tumor incidence in comparison with the natural background of $^1/_4$ to $^1/_5$ cancer-related deaths. It is interesting to note that the European Economic Community (COM(85)669 def., Bruxelles, December 3, 1985) is considering the possibility of putting the limit of occupational exposure at 5 ppm instead of 10 ppm, as generally accepted by different countries.

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