REVIEW OF THE CALIFORNIA DEPARTMENT OF HEALTH SERVICES RISK ASSESSMENT OF BENZENE

Prepared for

The Western Oil and Gas Association

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EXECUTIVE SUMMARY

- The risk assessment of benzene conducted by California's Department of Health Services (DOHS) differs substantially from risk assessments of benzene that have ben conducted by others, and several of the procedures and assumptions used by DOHS are without substantial justification.

- Although DOHS has relied heavily on portions of the analyses of carcinogenic risks of benzene prepared by expert groups of the International Agency for Research on Cancer and EPA's Carcinogen Assessment Group, DOHS selectively departs from the analyses of these expert groups in its reliance on animal carcinogenicity data for low-dose risk assessment, despite the large additional uncertainties this adds to the estimates of risk.

- DOHS's suggestion that the risks predicted at low doses based on the human and animal data are "remarkably close" does not take into consideration the substantial qualitative differences in the estimated risk because of diferences in species, route of exposure and temporal pattern of exposure.

- DOHS's use of just a single, highly conservative low-dose extrapolation model does not reveal the degree of uncertainty in risk estimates attributable to model selection when several models may be equally appropriate, based on statistical fit and mechanistic compatability.

- DOHS does not address the relevance to human health of tumors at a site (the preputial gland) in mice that has no counterpart in humans, or the uncertainty associated with inferring human risks from such a response in animals.

- DOHS provides no substantial justification for its use of a body surface area scaling factor for dose rate when the best available empirical evidence indicates that body weight is a more appropriate scaling factor than body surface area.

- DOHS's treatment of the available data on the mechanism of action of benzene is scanty, and the risk assessment would be improved if a more complete review of this important area was included.

- DOHS's risk assessment predicts unrealistically high estimates of human risk at exposure levels in the range of historical occupational exposure that are inconsistent with epidemiologic data. Specifically, the DOHS assessment predicts an excess risk of 0.23, almost one in four, for exposure to 10 ppm benzene (the current OSHA standard) 8 hours/day, 5 days/week, for 40 years. This is inconsistent with the data from epidemiologic studies.

- The various highly conservative procedures and assumptions used by DOHS when taken together substantially overestimate the risk to human health from exposure to benzene. Because of the uncertainty associated with any risk estimate, the degree to which risk is overestimated cannot be judged accurately, but is probably at least 24-fold and may be substantially more.

I. INTRODUCTION - PURPOSE OF REVIEW

The Western Oil and Gas Association (WOGA) requested that Environ Corporation conduct a review of the California Department of Health Services (DOHS) risk assessment of benzene. In this risk assessment, DOHS attempts to estimate possible lifetime risks of cancer to people exposed continuously for their lifetime to low levels of benzene in the air.

This report contains our evaluation of the DOHS risk assessment. Our evaluation is divided into two major sections. First, we address several broad issues raised by the procedures used by DOHS in conducting its risk assessment, particularly concerning its use of the available epidemiologic evidence regarding benzene's carcinogencity. In the second section, we address more specific questions raised by statements made in the DOHS assessment. These are organized sequentially according to where in the DOHS document the issue first arises.

Following these two major divisions of our report is a final section summarizing our findings and stating our conclusions regarding the scientific adequacy and utility of the DOHS risk assessment of benzene.

We note that our report consists solely of a review of the DOHS risk assessment and does not itself constitute a risk assessment. Nor does it contain a review of the scientific literature on possible health effects of benzene. This has been extensively reviewed elsewhere (e.g., IARC 1982a, Laskin and Goldstein 1977), and we assume that the reader of this report is familiar with this literature.

II. BROAD ISSUES

A. Selective Reliance on Opinions of Other Expert Groups

The DOHS has apparently relied heavily upon the review of information related to carcinogenic risks of benzene prepared by the International Agency for Research on Cancer (IARC 1982a). The DOHS has also relied on the assessment of benzene risk prepared by EPA's Carcinogen Assessment Group (CAG) (Albert 1979). Although this is commendable, the DOHS seems to have selectively departed from the analyses of these agencies. Thus, the DOHS apparently does not accept, or even mention, IARC's risk assessment of benzene based on the epidemiologic data (IARC 1981, 1982b). Although IARC's initial draft risk assessment (IARC 1981) which estimated that "a working lifetime exposure to 10ppm of benzene would be likely to result in at least 17 cases of leukemia per 1000 exposed workers and that the number could be considerably greater" was not published, the published version (IARC 1982b) does note that the relative risk for leukemia is increased more than 20-fold in workers exposed to benzene at about 100ppm. In both cases, IARC considers only human data in estimating risks.

The DOHS did briefly describe EPA's risk assessment (Albert 1979) which was also based on human data, but they apparently rejected it in favor of risk estimates based on animal data. There are significant limitations in these (and other) risk assessments based on epidemiologic data. These limitations include uncertainty regarding the background risk of leukemia in the absence of benzene exposure, the shape of the dose-response relationship relating dose

of benzene to leukemia risk, the influence of temporal pattern of exposure on risk, the existence of a threshold for the effects of benzene and the influence of concurrent exposure to other substances on risk. However, these epidemiology-based assessments deserve more serious consideration than DOHS has given them. Indeed, all other assessments of potential human health risk from benzene, of which there are at lease nine we have seen, give far greater weight to human data. Although the use of human data has the limitations described above, they have the important advantages that they involve both the species (man) and route of exposure (inhalation) in which we are interested. The use of data from animal studies introduces considerable additional uncertainty into any risk assessment. This has been concisely described by the Occupational Safety and Health Administration (OSHA 1980) in its cancer policy:

> Extrapolation from animal data to predict risks in humans introduces many additional uncertainties. These include selection of appropriate scaling factors for size, lifespan, and metabolic rate; differences in routes of exposure, duration and schedule of exposure, absorption, metabolism, and pharmacokinetics; differences in intrinsic susceptibility and repair capabilities; intra-population variation and susceptibility; and exposure to other carcinogens and intrinsic and extrinsic modifying factors. At least theoretically, these factors can affect the relative response of humans and animals by many orders of magnitude.

It would thus seem appropriate that DOHS give much more attention to the various estimates of risk projected from the several available epidemiology studies and to the advantages and disadvantages of using these risk estimates in decision-making.

B. Comparability of Risks Estimated from Human and Animal Data

It is not at all clear, as DOHS asserts, that the risk at low levels of exposure to benzene estimated from human data "is remarkably close to the risk

predicted from animal studies." Although the quantitative probability estimates are, indeed similar, the probability estimates derived from animal data by DOHS are based on conditions of exposure (gavage treatment with benzene in corn oil, once per day, 5 days per week for a large proportion of the lifespan starting at 6.5-8.5 weeks of age) and responses (preputial gland carcinomas) that are quite different in nature from those pertaining to the human data. Making statements about the comparability of quantitative probability estimates when these estimates are based on qualitatively very different conditions and responses is highly questionable. Moreover, the uncertainties in the two types of estimates are based on totally different factors. To use an old cliche, it is like comparing apples and oranges. In this situation the similarity between the magnitude of the probability estimates can be considered only as a coincidence.

C. Use of Just a Single Low-Dose Extrapolation Model

The use of just the multistage model for high-to-low dose extrapolation of animal data does not reveal the degree of uncertainty in risk estimates associated with model selection. We are not suggesting that the multistage model is inappropriate; in fact it is one of the most appropriate of the various models available for low-dose extrapolation. However, it is not the only appropriate model, and other models that are also justifiable may predict quite different risk estimates. In particular, some data sets may be more influenced by the choice of extrapolation model than others, particularly any data sets that show a highly curved (nonlinear) dose-response relationship. The sensitivity of the risk estimates to model selection should be revealed in quantitative terms, not simply by some broad qualitative statements.

D. Relevance of Mouse Preputial Gland Tumors to Human Health Risk

We have recently reviewed several benzene risk assessments, all of which place greater weight on human data than on animal data. Moreover, none of the treatments of animal data relies upon the same endpoint (preputial glands tumors in male mice) that has been used by DOHS. There are, admittedly, no "standard" criteria for selection of tumor data sets for extrapolation to humans, so that is is difficult to provide well-established precedents for this choice. The DOHS's sole justification for their selection appears to be that it yields the highest value of unit risk. It would appear that DOHS should at least discuss the biological relevance of the tumor to humans, and the possible greater relevance of alternative choices of tumor data. The preputial glands in rodents are modified sebaceous glands located in the subcutaneous tissue near the tip of the penis (Squire et al. 1978). They have no direct counterpart in humans and DOHS has made no attempt to link the considerable data on the toxicity of benzene in humans with the observation of these rare tumors in mice. Although DOHS is correct in pointing out that the tumor type or target organ is often different in different species with the same carcinogen and that the available epidemiologic studies might not have detected increases in certain common tumors (e.g., lung, mammary gland) in humans, it is likely that they would have detected rare tumors. There seems little justification for supposing that the production of rare tumors in mice would predict one of these common tumors in humans. DOHS should give more consideration to this question of the relevance of rodent preputial gland tumors to humans.

E. Use of Surface Area Adjustment for Interspecies Scaling

Apart from a comment that scaling the dose rate by body surface area is "a procedure routinely used by pediatricians for calculating medical doses for babies and children," DOHS provides no justification for its use of this procedure. Although mg/m^2 appears to give a better correlation for the acute toxic effects of drugs, and as DOHS points out is widely use for scaling dosages of drugs, the relevance of a scaling procedure appropriate to acute toxic or pharmacologic effects of drugs to the situation of lifetime exposure to a carcinogen is not clear, and the best basis for deciding on a scaling procedure is empirical evidence. In fact, the available empirical data comparing doses corresponding to equal risk in humans and experimental animals, for those chemicals known to cause cancer in both, reveal that the dose scaling units giving the closest correlation between humans and animals are mg/kg body weight/day, not mg/m^2/day (Crump et al. 1980).

F. Consideration of Mechanism of Benzene-Induced Leukemia

DOHS has apparently accepted EPA's view of the available data on the mechanism of benzene-induced leukemia; specifically the existence or non-existence of a threshold for the carcinogenic effect of benzene. However, DOHS's treatment of this issue is scanty and consists largely of a brief summary of opinions expressed by EPA (USEPA 1984). While DOHS's basic conclusion, that definitive evidence for the existence of a threshold for benzene has not been produced, may be correct, it would be valuable if it were more fully reviewed and documented, and if the uncertainty in the conclusion

were explicitly discussed.

G. Implications of Risk Estimates to Exposures in the Range of Occupational Exposures

DOHS estimates a risk in addition to background of "1700 x 10^{-6}" from lifetime exposure to 10 ppb benzene. This implies an excess risk of: $1-\exp[-(1700 \times 10^{-6} \times 1000)] = 0.82$ for lifetime exposure to 10 ppm, the current OSHA standard level, and at the low end of the range of exposures in the past. Even if we adjust for the fact that occupational exposure is not continuous for a lifetime, the predicted excess risk is still so great (0.23, assuming exposure 8 hours/day, 5 days/week, for 40 years) that it is inconceivable that so high a risk would not have been detected in the epidemiologic studies; this would correspond to about a doubling of the incidence of all forms of cancer. Such an effect has not been seen even in cases with generally higher exposure levels. While we recognize that the DOHS estimate is intended as an upper limit on risk, the unrealistically high risk levels that it predicts, which are inconsistent with historical occupational data, demonstrate that the DOHS estimate substantially overestimates the risk to humans at low exposure levels. This estimate is higher than the range predicted by earlier risk estimates of 0.00058 to 0.13 for the same exposure.

H. Sensitivity of Risk Estimates to Changes in Assumptions and Procedures

As we have pointed out DOHS uses several assumptions and procedures without adequate documented justification. These can substantially affect the

quantitative estimate of human risk. For example, the assumption that risk in different species is equal at the same daily dose measured in mg/m^2 body surface area (or 2/3 power of body weight) results in predicted human risks derived from the NTP mouse data that are about 12-times greater than risks predicted on the basis of risk equivalence at doses measured in mg/kg body weight/day for which there is greater empirical evidence (Crump et al. 1980).

The use of the upper 95th precentile confidence limit on risk rather than the maximum likelihood estimate (i.e., the estimate that best fits the experimental data) further elevates the DOHS estimates of human risk by a factor of about twofold to threefold. Further, by relying solely on the linearized multistage model for low-dose extrapolation, DOHS estimates "risks" to humans that are higher than would be predicted by other models. As we have already pointed out, DOHS should investigate the effects of the use of different low-dose extrapolation models on its predicted risks.

III. SPECIFIC COMMENTS

The following comments are listed by page and paragraph of the DOHS document. Some are minor, but several suggest that considerable additional review could be usefully undertaken by the Department.

Page 4, second paragraph: In their discussion of a 1953 metabolism study of radioactively-labelled benzene, DOHS noted that 5-10% of an orally administered dose of benzene that was not recovered in the "2-3 days" of the study was "bound to body tissues." However, although 5-10% of the administered radioactivity may not have been eliminated in the 2-3 days of the study, it is not at all clear that it was "bound to body tissues." It is not unusual for 5-10% of the administered radioactivity to remain unaccounted for in a metabolism study of this vintage. To attribute this to tissue-binding is not justified.

Page 6, complete paragraph: In this paragraph, DOHS notes that rabbit bone marrow is capable of metabolizing benzene to oxidized forms. Although it may be reasonable to assume that this is also the case in humans, DOHS presents no evidence that this has been demonstrated experimentally with human bone marrow and does not explicitly note that it assumes that benzene metabolism also occurs in human bone marrow.

This paragraph also provides an example of DOHS's selective departure from the analysis of IARC, since DOHS fails to note IARC's statement that "the observation...that partial hepatectomy protects against benzene toxicity suggests that a metabolite formed in liver is essential for benzene toxicity."

Page 8, first complete paragraph: DOHS notes, without providing any support, that there is "convincing evidence of a role for polyhydroxy metabolites in benzene toxicity." Not only does this statement require expanding and a description of the "convincing evidence" but it also requires reconciling with statements made elsewhere in the DOHS document (e.g., in Figure I.1, and on p. 5) implicating benzene oxide as the toxicant.

Page 8, third-to-last sentence: At one point in this sentence DOHS talks about binding of benzene metabolites to "the nucleic acid fraction" and at another point mentions binding to "nucleic acids". There are important differences between "nucleic acids" and the "nucleic acid fraction" since, depending on the fractionzation procedure used, the nucleic acid fraction of a tissue may contain material (e.g. histones) in addition to nucleic acids.

Page 9, first paragraph: DOHS states that "benzene is rapidly absorbed after inhalation." However, it is important to note that, as DOHS states elsewhere (p.1), only about 50% of inhaled benzene is retained.

Page 9, last sentence: DOHS states that "several studies have demonstrated covalent binding by an unidentified benzene metabolite." However it is important to note that only one study (Lutz and Schlatter 1977) has specifically identified DNA as a site of binding.

Page 11, last paragraph: DOHS appears to make a point of noting positive mutagenicity data on ozonation products of phenol. However, this test material has no relevance to human exposure to benzene because ozonation products of phenol have not been detected as, and are not likely to be, metabolites of benzene in humans or animals.

Page 12-13, carryover paragraph: DOHS describes work with a lymphoblastoid cell line said to have an endogenous metabolic activation system. The metabolic capability of this cell line is not well documented and the results reported with benzene (about a twofold increase in mutation frequency with a single dose of benzene) are of questionable biological significance. We agree with DOHS's description of these data as "very limited."

Pages 13-14, entire section on DNA damage: The only evidence that DOHS cites for the ability of benzene to cause DNA damage is evidence that it induces sister chromatid exchanges (SCEs). However, induction of SCEs is not definitive evidence of induction of DNA damage. The mechanism of production of SCEs is not known. Although DNA strands must presumably be broken and rejoined to produce an exchange, the effect of some SCE-inducers may be to interfere with the rejoining of naturally occurring strand breaks rather than to cause DNA damage (strand breaks).

Page 16, first paragraph: DOHS describes some work in which "the authors urged the scoring of "gaps" in studies of chromosomal damage." The reason gaps are often not scored in chromosome aberration studies (or at least counted separately) is that it is unclear if they represent damage to DNA or an effect on the protein component of the chromosomes that affects normal staining, since gaps show no clear evidence of breakage of the DNA strand.

Page 18, first paragraph: DOHS describes a cytogenetic study of workers employed in distilling coal tar. Although DOHS notes that "exposures to chemicals other than benzene may also have occurred," there is a definite implication that benzene was the causative agent. In fact there are many

chemicals other than benzene that workers distilling coal tar would be exposed to and that might cause chromosomal aberrations. Attributing the effect to benzene is highly questionable.

Page 19, last sentence: The meaning of this sentence ("In this study no assessments of dose-response relationships were made due to the limited number of colonies scored for any one test chemical.") is completely unclear.

Page 20, last sentence: DOHS again cites induction of SCEs as evidence of DNA damage. As discussed above, SCEs do not represent definitive evidence of DNA damage.

Page 28, last paragraph: DOHS suggests that "women may be more susceptible to benzene toxicity than men," because of greater body fat storage of benzene. However, the greater fat storage of benzene by women may protect them from toxicity rather than make them more susceptible since fat is not the target for benzene. With more benzene in body fat, the concentration in bone marrow (the target organ) is likely lower in women than in men exposed to the same total amount of benzene.

Page 29, first and second paragraphs: DOHS cites a Russian study of menstrual and reproductive disorders among "female gluing operatives in a mechanical rubber product factory who were exposed to petroleum and chlorinated hydrocarbons, petroleum being a major source of benzene." Based on the brief description given of this study, it is not clear if the study was adequately conducted with properly matched controls. Also, the women in this study were apparently exposed to chemicals other than benzene. Hence, attribution to

benzene of any effects seen is without sufficient justification.

Page 29, last paragraph: DOHS describes a small case-control study of children with nervous system defects and notes that the mothers of these children were exposed to "organic solvents" more than were control mothers. Attribution of these effects to benzene exposure is quite unjustified. In fact, it is unclear from the information cited whether any of the mothers were exposed to benzene per se; only "organic solvents" are specified by DOHS.

Page 30, First paragraph, last two sentences: DOHS concludes that because mixed function oxidases (MFOs) appear at an earlier stage in gestation in humans than they do in the rat, "the human fetus is [sic] exposed to the mutagenic and carcinogenic properties of benzene oxide for a greater part of its development gestation [sic] than the rat fetus." MFOs are a class of related enzymes involved in the oxidation of organic chemicals. Many different MFOs are found, each with a different substrate specificity. While some MFOs may appear earlier in human gestation than in rat gestation, DOHS has not demonstrated that the particular MFOs that metabolize benzene to benzene oxide are present at this early stage. Even assuming that the MFO's that appear early in human gestation are capable of metabolizing benzene, it would be more appropriate to say that "the human fetus may be exposed..." rather than "...is exposed..."

Page 30, second paragraph, fourth sentence: The evidence cited that benzene can affect menstrual and reproductive function is highly questionable because of many confounding factors (see comments regarding p. 29, first and second paragraphs).

Page 38, first paragraph, last sentence: DOHS states that "the case reports established the relationship between benzene and leukemia, principally acute myelogenous." By their nature, case reports alone can not establish the relationship between benzene and leukemia. Case reports by themselves may establish the hypothesis of a relationship, or may suggest a relationship. More complex epidemiologic investigations, such as case-control studies or cohort studies are needed to establish a relationship.

Page 42, first complete paragraph, last sentence: DOHS suggests that "a larger epidemiological study might indeed implicate benzene in the causation of cancer at sites other than the hematopoietic system." It would be more accurate to say that a larger epidemiological study would be useful to determine whether there was a relationship between benzene exposure and neoplastic diseases other than leukemia.

Page 43, second paragraph: DOHS suggests that occupational studies underestimate the risks of benzene to the general population because women and children would receive a larger dose of benzene from the same air concentration because of their lower body weight. This statement is in general true, as long as all individuals considered are performing similar degrees of physical work in relation to their body size. The dose received by inhalation depends on work rate as well as body size, since work rate is proportional to O_2 consumption and inhalation rate. If the workers studied were all doing heavy work, their dose of benzene would be greater than that received by women or children resting or doing light work.

Page 54, second paragraph, fourth sentence: This sentence, "This will be reflected in a slope parameter that is also too low," is incorrect. If as DOHS states, the expected number of cases of leukemia is underestimated, the ratio of observed/expected cases will be higher than it should be and the slope parameter will be overestimated, i.e., too high, not too low.

Page 59, halfway down table in "Observations" column: The meaning of the statement, "major survival of animals of control group" in this table is unclear.

Page 60, last sentence: The parenthetical description of tumors induced by benzene "(Zymbal gland of the oral cavity...)" is meaningless as written; the Zymbal gland is in the ear. Presumably what is meant is "(Zymbal gland and the oral cavity...)."

Page 61 and 62: In this tabulation of the results of studies of benzene by Maltoni, tumor incidences are reported only as differences between treated and control groups. It would be more informative if the actual incidences in control and treated groups were presented. The same criticism applies to the tabulation of results of the NTP bioassay on pages 66 and 67.

Page 65, last complete sentence: DOHS suggests that the studies by Maltoni and NTP show that the female rat is less sensitive than the male to benzene-induced Zymbal gland carcinomas. This is incorrect. Although the incidence of Zymbal gland carcinomas in high dose male rats (17/42) was greater than that in high dose female rats (14/46) the dose of benzene

received by the males was twice that received by the females. At the same dose level (100 mg/kg) the incidence in males (10/42) was lower than in females (14/46) and the difference is even greater after subtraction of the background incidence (males 18%, females 30%). Also, in the studies of Maltoni, females appeared as sensitive as or more sensitive than males to the induction of Zymbal gland carcinomas (Maltoni and Scarnato 1979, Maltoni et al. 1982, Maltoni et al. 1983).

Page 80, first paragraph: DOHS notes that it uses a body surface area scaling factor for extrapolating from animals to humans, but provides no justification for the use of such a scaling factor. As discussed in Section II.E above, empirical evidence does not support the use of a surface area adjustment of dosage. Analysis of carcinogenicity data for the same chemicals in humans and experimental animals reveals that the mg/kg/day dosage unit provides the closest comparison between species (Crump et al. 1980).

Page 80, third paragraph: DOHS notes that it assumes that the lifetime average daily dose is the critical measure in assessing risk from benzene but provides little discussion of the issue. This issue requires greater attention since there is substantial evidence that, at least for some chemicals, the timing of exposure to a carcinogen is critical in determining the risk. Depending on the stage of the carcinogenic process at which a chemical acts, the same total dose of a carcinogen may cause quite a different tumor incidence depending upon the time of life when exposure occurs (Day and Brown 1980).

Page 81, second paragraph, first sentence: The phrase "adipose secretory gland cancers" is rather obscure. If it refers to Zymbal gland and preputial gland carcinomas, it would be clearer and just as correct to refer to "modified sebaceous gland cancers."

Page 83-85, carryover paragraph: The logic of this paragraph is rather hard to follow. DOHS starts by raising the issue of increased mammary tumors in mice and rats, and then talks about the possible increase in lung tumors in epidemiologic studies of benzene. This might support the consideration of the increase in lung tumors seen in mice, but DOHS does not mention this. DOHS should provide more scientific justification for its choice of tumor incidence data for human risk extrapolation of tumors arising at a site that does not exist in humans (the preputial gland) following administration of benzene by a route (corn oil gavage) not related to the most likely route of human exposure (inhalation).

Page 85, last complete sentence: The meaning of this sentence, "In this context the exposure to 1 to 10 ppb of benzene in ambient air and its attendant risk is not great in comparison to occupational and indoor air exposure to benzene or in comparison to the 25% lifetime risk of cancer run by the average American (Leven et al., 1974)" is obscure. Although it is clear that the risk from benzene is less than 25%, the average lifetime risk of any form of cancer, the rest of the sentence is less clear. Is DOHS suggesting that the general population is exposed to higher concentrations of benzene indoors than outdoors?

Page 87, complete paragraph, third sentence: In this sentence, "When one considers that airborne benzene affects tens of millions of individuals in the urban parts of California...", the use of the word "affects" may be misleading. This sentence could easily be misconstrued as saying that tens of millions of individuals will be "affected" by cancer (or leukemia) caused by benzene. It would be clearer to say that this many people may be exposed to low levels of benzene in ambient air. Whether they are "affected" by this exposure can only be answered in terms of a highly uncertain probability estimate arising from the risk assessment.

Page 88: In its listing of assumptions used in its risk assessment, DOHS notes that it uses a lifetime-average body weight of 60kg (point 1.1), but in point 1.3, DOHS makes no attempt to average air intake over the lifetime as it should to make air intake comparable to body weight. Also, DOHS provides no justification for its statement in point 1.7 that "a scaling factor based on surface area provides the best estimation of equivalent doses between species." As discussed in Section II.E, above, the dose units of mg/kg/day provides the closest correlation between species in sensitivity to carcinogens (Crump et al. 1980).

Page 89: DOHS's conversion of CAG's unit risk from units of $(mg/kg/day)^{-1}$ to ppb^{-1} is correct only if CAG originally assumed 100% absorption of benzene in calculating its unit risk. It is unclear if this was the case. The original CAG risk assessment (Albert 1979) derived a slope parameter in units of ppb^{-1}. The source of the updated unit risk provides no description of how the value was derived.

Page 94, section b.1: DOHS provides no support for its statement that the dose in mg/kg of benzene in an inhalation study in animals is proportional to O_2 consumption and (body weight)$^{2/3}$.

Page 10, first and third lines: The values of multistage model parameters a_i, b_i and q_i are described by DOHS as being greater than zero, but they may also be equal to zero; the only restriction is that they are not negative.

Pages A4 A5, carryover sentence: DOHS's statement that "significant excesses of kidney and thyroid tumors were produced both in rats and mice at dose levels that did not produce cellular damage in an NCI bioassay on chloroform" is incorrect. There was no increase in kidney or thyroid tumors in mice and no significant increase in thyroid tumors of any single histological type in rats; only if the unrelated thyroid C-cell tumors and follicular cell tumors were combined would there be a "significant" increase. However combining these different types of tumors is not an acceptable procedure because the different cell types have different functions and are of different embryonic origins. Further, although NCI did not report an increased incidence of nonneoplastic kidney lesions in treated rats, the depression in body weight and reduction in survival in the treated animals demonstrates that some toxic effects were produced, and the high incidence of nonneoplastic kidney lesions that normally develop in about 75% of aging male rats and 36% of aging female rats of the strain used (Goodman et al. 1980) would likely have obscured any subtle effect of chloroform.

IV. SUMMARY AND CONCLUSIONS

The risk assessment of benzene conducted by California's DOHS deviates significantly from all others we have seen, particularly in its reliance on animal carcinogenicity data for low-dose risk estimation to the virtual exclusion of the extensive human data. DOHS's rationale for this procedure is that the epidemiologic data may underestimate the risk, since possible tumor sites other than the hematopoietic system have not been rigorously studied. However, to exchange this uncertainty for the uncertainties associated with interspecies risk extrapolation based on tumors of a tissue that does not exist in humans, induced following administration of benzene by a route (gavage) unrelated to the most likely route of human exposure (inhalation), seems extremely questionable. And to suggest that the risks predicted by the human data are similar to those predicted by the animal data because the probability estimates are close, does not take into consideration the wide qualitative differences in the predicted risks and the fact that the uncertainties in the two estimates derive from totally different factors. Further, the unrealistically high excess risks the DOHS assessment predicts for exposure in the range of past occupational exposure demonstrate that DOHS overestimates the risk at low-dose levels.

In general, the DOHS document suffers from a lack of adequate, documented justification for several of the procedures it incorporates and conclusions it reaches, particularly where it departs, selectively, from the procedures and conclusions of expert groups such as IARC and CAG.

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ATTACHMENT 2

EMBRYOTOXICITY OF INHALED BENZENE IN MICE AND RABBITS

By

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Summary

The effect of inhaled benzene on embryonal and fetal development was assessed in mice and rabbits. CF-1 mice and New Zealand white rabbits were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Little evidence of maternal toxicity was discerned in either species. Exposure to benzene altered the incidence of some minor skeletal variants among the offspring of both species. In mice, a significant decrease in fetal body weight was noted. Although some evidence of embryotoxicity was seen in both species, a teratogenic effect was not observed in either mice or rabbits inhaling 500 ppm of benzene.

INTRODUCTION

Benzene (benzol, C_6H_6) is used primarily as a starting material in the production of a wide variety of organic chemicals. Benzene is also used as an industrial solvent and as an anti-knock agent in gasoline. Acute exposure to benzene vapors produces central nervous system depression (Browning, 1965). Occupational exposure to high levels of benzene on a chronic basis has been associated with leukemia (Aksoy et al., 1972a; 1974; Berlin, 1974), aplastic anemia (Saita, 1973; Aksoy, 1972b), and chromosomal aberrations (Pollini and Colombi, 1964; Vigliani and Forni, 1969; Forni et al., 1971). The OSHA-proposed standard for an occupational exposure to benzene for 8 hours per day, 5 days per week is 1 ppm (OSHA, 1977).

Several studies have been conducted in laboratory animals to evaluate the potential teratogenicity of benzene. Watanabe and Yoshida (1970) reported cleft palate, agnathia, and micrognathia among the offspring of mice given a massive subcutaneous injection of 3 ml benzene/kg body weight on day 13 of gestation. In another study, pregnant rats were exposed to 0, 10, 50, or 500 ppm of benzene for 7 hours per day from days 6 through 15 of gestation (unpublished data,

Hazleton Laboratories Corp., Vienna, Va.). A low incidence of malformations (exencephaly, angulated ribs, and non-sequential ossification of forefeet) was observed among the litters of rats exposed to 500 ppm, suggesting a possible teratogenic effect at that concentration. In contrast, Green et al., (1978) found no evidence of a teratogenic effect in Sprague-Dawley rats inhaling 300 or 2200 ppm of benzene for 6 hours per day from days 6 through 15 of gestation.

The purpose of the study presented herein was to assess the effect of inhaled benzene on embryonal and fetal development in two additional species. For these studies, pregnant CF-1 mice and New Zealand white rabbits were exposed to 500 ppm of benzene by inhalation for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Since the development of leucopenia has been demonstrated in rats, guinea pigs, and rabbits following repeated exposure to benzene vapors (Wolf et al., 1956), blood samples from the female mice and rabbits and from rabbit fetuses were collected for hematologic evaluation. This work was sponsored jointly by Exxon Corporation and The Dow Chemical Company.

MATERIALS AND METHODS

Test Material. Benzene, identified as ID95-01000-0070, was supplied by Exxon Chemical Company U.S.A., Houston, Texas. The test sample was analyzed at the Analytical and Information Division, Exxon Research and Engineering Company, Linden, New Jersey (Table 1). On at least two days of exposure, however, the test animals were exposed inadvertently to a sample of benzene obtained from Mallinckrodt Chemical Company; the Mallinckrodt benzene was identified as nanograde quality, Lot No. 1043B3. Samples of both the Exxon and Mallinckrodt benzenes have been retained for possible comparative analysis.

Test Animals. Virgin CF-1 mice (Charles River, Portage, Michigan) and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) were housed in wire mesh cages in a room controlled for temperature (70±3°F), humidity (45±5%), and light cycle (12 hrs light and dark). The animals were maintained on commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri) and tap water free choice except while in the exposure chambers. Mice and rabbits were allowed at least two and three weeks, respectively, for acclimatization to the laboratory before use. The day on

ATTACHMENT 3

5 Benzene

ENV - P.C.

Need data on:
Carcinogens -
Benzene

TERATOLOGY STUDY IN RATS

BENZENE

FINAL REPORT

Submitted to

Exxon Research and Engineering Company
Linden, New Jersey

HAZLETON LABORATORIES AMERICA, INC.
9200 Leesburg Turnpike • Vienna, Virginia 22180 • U.S.A.

September 25, 1975

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HAZLETON LABORATORIES AMERICA, INC.

SPONSOR: Exxon Research and Engineering Company DATE: September 25, 1975

MATERIAL: Benzene

SUBJECT: FINAL REPORT
Teratology Study in Rats
Project No. 145-552

SUMMARY

This study was designed to evaluate the teratogenic potential of Benzene when administered to female rats by inhalation from Day 6 through Day 15 of gestation at levels of 0, 10, 50, and 500 ppm.

Criteria evaluated for compound effect included maternal body weights, weight gains, appetite, appearance and behavior, hematology values, pregnancy rates, survival rates, and reproduction data and offspring viability and growth.

No effects attributable to the administration of Benzene were noted in comparisons of appearance and behavior, survival rates, hematology values, pregnancy rates, or implantation efficiencies. In addition, no compound-related differences were noted in evaluations of fetal viability or sex.

The mean maternal body weight gains of the mid and high dose groups were lower than that of the control group during the treatment period. Although once treatment was discontinued, the animals of these groups gained weight at a rate exceeding that of the control animals, corresponding statistically significantly lower mean fetal body weights (mid and high dose groups) and crown-rump distances

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(high dose group only) and decreases in the degree of ossification were noted in the fetuses derived from the maternal females of the mid and high dose groups. The maternal weight suppression and the related fetal growth retardation noted in these two groups were attributed to Benzene exposure.

Postmortem evaluation of fetuses revealed lagging skeletal ossification among the mid and high dose groups reflecting overall growth retardation of both dams and fetuses. However, the four cases of skeletal malformations (exencephaly - one case; angulated ribs - one case; and non-sequential ossification of forefeet - two cases) suggested a possible teratogenic effect due to Benzene inhalation at 500 ppm.

INTRODUCTION

This study was designed to evaluate the embryotoxic and/or teratogenic effects of Benzene when administered by inhalation to female rats from Day 6 through Day 15 of gestation. Exposure of the animals was initiated on May 6, 1975, and sacrifice of the dams for cesarean delivery was completed on June 5, 1975.

TEST MATERIAL

The test material, Benzene, was a clear, colorless, highly flammable liquid with an odor resembling gasoline. The material was received from the sponsor on April 15, 1975 in four 5-gallon cans.

TEST ANIMALS

Eighty Sprague-Dawley cesarean-derived female rats received from Charles River Laboratories, and forty stock male rats of the same strain were used for this study.

METHODS

Breeding

Two female rats were placed in a wire mesh breeding cage with each male. A vaginal smear was taken from each female daily and examined for the presence of sperm. If sperm was observed, the female was assumed to have mated and was removed from the breeding regimen and housed separately. The day on which evidence of mating was observed was designated as Day 0 of gestation. Females not observed to have mated within 21 days were discarded.

Assignment to Groups

Twenty females were arbitrarily assigned to each group. Of these 80 rats, 74 were assumed to have mated and their dispersion among groups is as follows:

Group	No. of Animals	Exposure Level ppm
1 (control)	17	0
2 (low)	18	10
3 (mid)	20	50
4 (high)	19	500

ATTACHMENT 4

Toxicology, 11 (1978) 55–63

EMBRYOTOXIC EFFECTS OF BENZENE AND ITS METHYL DERIVATIVES: TOLUENE, XYLENE

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SUMMARY

CFY rats were exposed to inhalation of 1000 mg/m^3 (313 ppm) benzene, 1500 mg/m^3 (399 ppm) toluene, or 1000 mg/m^3 (230 ppm) xylene for 24 h/day from day 9 to day 14 of pregnancy; to that of 1500 mg/m^3 (399 ppm) toluene for 24 h/day from day 1 to day 8 of pregnancy, or 1000 mg/m^3 (266 ppm) toluene for 8 h/day from day 1 to day 21 of pregnancy. CFLP mice were exposed to inhalation of 500 mg/m^3 (133 ppm) toluene for 24 h/day from day 6 to day 13 of pregnancy. Untreated animals and groups inhaling pure air served as controls.

None of the solvents proved to be teratogenic, the incidence of malformations did not change as a result of exposure, though an increase in skeletal anomalies (extra ribs, fused sternebrae) was observed with all 3 solvents. Benzene and toluene also caused considerable retardation of fetal development.

The growth retarding effect of toluene on fetal development in early pregnancy is of particular importance from the point of view of occupational hygiene.

INTRODUCTION

The testing of industrial chemical agents for mutagenicity, carcinogenicity and teratogenicity is an important task of present-day toxicology and occupational hygiene. Such studies have so far been rather unsatisfactory in respect of benzene, toluene and xylene.

Most of the data available concern the mutagenicity of these agents. The

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damaging effect on chromosomes of benzene has been described in workers exposed to benzene [1—6]. A similar effect of benzene on human leukocytes in vitro has been reported [7]. Forni et al. [3] failed to find chromosome abnormalities in workers exposed to toluene. Dobrokhotov [8], Lyapkalo [9] and Dobrokhotov and Enikev [10] reported on chromosome abnormalities in the bone-marrow cells of rats treated with benzene and toluene.

The carcinogenic effect of benzene has been amply proved. More than 100 cases of leukaemia that were attributable to benzene exposure were described in the past 40 years [11]. Mazucco [12] treated the skin of mice with carcinogenic agents and obtained a shortened latency and decreased skin collagen content when using benzene or toluene as carrier substance.

Little is known about the tetrogenicity of benzene and its homologues, although these solvents have been included in the list of chemicals suspected to have such an effect [13,14]. Watanabe and Yoshida [15] treated rats with s.c. benzene and found cleft palate, agnathia, micrognathia and other skeletal malformations in the animals. A connection between the occurrence of sacral aplasia and occupational exposure to organic solvents has been demonstrated by Kucera [16], who also produced a developmental malformation analogous to sacral aplasia with xylene treatment in chick embryos. Chebotar [17] did not find any developmental defects in fetuses of rats exposed to xylene inhalation.

The wide-spread application of benzene and its homologues in industry, also in plants employing many young women in the reproductive age, makes it imperative to clear up the tetratogenicity and embryotoxicity of these solvents.

The teratogenic and embryotoxic effects of benzene, toluene and xylene inhalation were studied experimentally, devoting most attention to toluene which is especially widely used. A comparison of the 3 solvents was also made.

MATERIALS AND METHODS

CFY female rats weighing 240—280 g and CFLP female mice weighing 28—30 g were used. Procedures of mating and housing of the animals as well as the parameters of the inhalation chambers are described in our preceding paper [18]. The pregnant animals inhaled benzene*, toluene*, xylene** or clean air according to the protocol of the experiment shown in Table I. Solvent concentration in the chambers was checked using a Hewlett-Packard gas chromatograph.

The rats were sacrificed on day 21 of pregnancy, the mice on day 18. The fetuses were processed and the results were analysed statistically according to the methods described previously [18].

* Analytical purity, Biogal, Debrecen.
** (10% *o*-xylene, 50% *m*-xylene, 20% *p*-xylene, 20% aethylbenzene), Reanal, Budapest.

TABLE I

LAYOUT OF THE EXPERIMENT AND SUMMARIZED DATA OF THE EXPERIMENTAL GROUPS OF BENZENE, TOLUENE AND XYLENE TREATED PREGNANT ANIMALS

Experimental groups			No. of pregnant animals/group		Maternal[a] weight gain (%)	No. of fetuses			Fetal loss[b] (%)	Mean litter size	Mean fetal weight (g)	Mean placental weight (g)	Weight retarded fetuses[c] (%)
Species	Time of inhalation	Substances and doses	Examined	Died		Live	Resorbed	Dead					
Rats	Untreated control	—	28	—	52.45± 1.23	315	10	1	3.37	11.25± 0.54	3.83± 0.02	0.51± 0.005	2.9
	9—14 Days of pregnancy	Air	26	—	46.86± 2.04	348	15	—	4.13	13.38± 0.50	3.76± 0.02	0.47± 0.004	6.9
	24 h/day	Benzene 1000 mg/m³	19	—	22.54± 5.98**	226	12	4	6.60	11.89± 0.89	3.38± 0.02**	0.47± 0.006	38.0†
		Toluene 1500 mg/m³	19	2	41.75± 2.43	213	18	—	7.79	11.21± 0.54	3.75± 0.03	0.46± 0.005	17.3
		Xylene 1000 mg/m³	20	—	46.20± 2.01	286	15	1	5.30	14.30± 0.57	3.75± 0.02	0.51± 0.003	5.0
	1—21 Days of pregnancy	Air	10	—	46.58± 2.34	111	8	—	6.72	11.10± 0.59	3.81± 0.03	0.53± 0.006	7.2
	8 h/day	Toluene 1000 mg/m³	10	—	44.07± 2.37	133	3	—	2.21	13.30± 0.56	3.61± 0.03	0.52± 0.006	16.0
	1—8 Days of pregnancy 24 h/day	Toluene 1500 mg/m³	9	5	44.03± 3.60	95	6	—	5.94	10.56± 1.30	3.31± 0.08**	0.53± 0.013	46.0†
Mice	6—13 Days of pregnancy	Air	14	—	—	124	6	1	6.10	9.00± 0.74	1.07± 0.01	—	6.5
	24 h/day	Toluene 1500 mg/m³	—	15	—	—	—	—	—	—	—	—	—
		Toluene 500 mg/m³	11	—	—	112	10	—	8.20	10.18± 1.06	0.96± 0.01**	—	27.6†

[a] In per cent of starting body weight.
[b] In per cent of total implantates.
[c] Per cent of living fetuses weighing less than 3.3 g (rats) or 0.9 g (mice).
± S.E.; ** $P < 0.01$ (*t*-test); † $P < 0.05$, (Mann Whitney U test).

RESULTS

There was no difference between the parameters of air inhaling and untreated controls. (Tables I and II).

Benzene

None of the rats exposed to 1000 mg/m^3 (313 ppm) benzene for 24 h/day from day 9 to day 14 of pregnancy died. The maternal body weight gain in per cent of the original body weight was lower in this group than in the air inhaling controls ($P < 0.01$) (Table I). There was no increase in the number of dead and resorbed fetuses. The mean fetal weight was significantly less than in the controls ($P < 0.01$). Close to 40% of the fetuses showed retarded development weighing less than 3.3 g (Table I). No external malformations were noted (Table II). As for visceral malformations thymus hypoplasia was found in 1 case, and dilatation of the urinary tracts in a number of cases, but with a frequency not surpassing that found in the controls. There was an increased occurrence of retarded skeletal development ($P < 0.05$). Skeletal examination showed a higher frequency of irregular, fused sternebrae and extra ribs ($P < 0.05$ for both) (Table II).

Toluene

The mice exposed to 1500 mg/m^3 (399 ppm) toluene inhalation died within 24 h. In the group of mice exposed continuously to 500 mg/m^3 (133 ppm) concentration from day 6 to day 13 of pregnancy the average number of fetuses did not differ from that in the air inhaling control group (Table I). The average fetal weight decreased and the proportion of weight retarded (less than 0.9 g) fetuses increased (Table I). No external or skeletal malformations were found (Table II). Visceral malformations, such as dilated urinary tracts, and signs of skeletal growth retardation occurred with the same frequency as in the controls (Table II).

21 rats were exposed to continuous inhalation of toluene in 1500 mg/m^3 (399 ppm) concentration from day 9 to day 14 of pregnancy. 2 of these animals died; in the surviving mothers body weight gain, fetal loss and the mean fetal and placental weights were found to be identical with those of the air inhaling group (Table I). External malformation — absence of tail — occurred in 2 of the 213 fetuses examined (Table II). Dilated urinary tracts were found in the same frequency as in the air inhaling controls (Table II). There was no difference in the occurrence of retarded skeletal growth either. Irregular sternebrae and extra ribs, however, occurred more frequently ($P < 0.05$ and 0.01, respectively) (Table II).

Of the 14 rats exposed to continuous inhalation of toluene in 1500 mg/m^3 (399 ppm) concentration from day 1 to day 8 of pregnancy, 5 animals died. The body weight gain of the survivors did not differ from that of the air exposed controls. No difference was found in respect of fetal loss either (Table I). The average fetal weight decreased ($P < 0.01$), that of the placentae did not. Approximately 50% of the fetuses were underweight

TABLE II

DATA OF THE FETUSES OF BENZENE, TOLUENE AND XYLENE TREATED PREGNANT ANIMALS

	Experimental groups									
	Rats								Mice	
	Untreated control	Air inhalation 9—14 days 24 h/day	Benzene inhalation 9—14 days 24 h/day	Toluene inhalation 9—14 days 24 h/day	Xylene inhalation 9—14 days 24 h/day	Air inhalation 1—21 days 8 h/day	Toluene inhalation 1—21 days 8 h/day	Toluene inhalation 1—8 days 24 h/day	Air inhalation 6—13 days 24 h/day	Toluene inhalation 6—13 days 24 h/day
No. of litters examined	28	26	19	19	20	10	10	9	14	11
No. of live fetuses	315	348	226	213	286	111	133	95	124	112
External malformations										
Agnathia	—	—	—	—	2	—	—	—	—	—
Brachimelia	—	—	—	—	—	—	—	—	1	—
Missing tail	—	—	—	2	—	—	—	—	—	—
No. of fetuses dissected	166	179	119	110	146	54	64	49	64	58
Internal malformations										
Anophthalmia	—	1	—	—	—	—	—	—	—	—
Hydrocephalus	—	—	—	—	—	—	—	4	—	—
Thymus hypopl.	—	—	1	—	—	—	—	—	—	—
Hydronephorosis	3	16	6	4	26	1	6	4	1	3
No. of Alizarin-stained fetuses	143	169	108	102	143	57	69	42	60	54
Skeletal retardation signs[a]	—	11	24†	6	17	—	17†	7†	3	1
Skeletal anomalies										
Fused sternebrae	1	2	13†	7†	8†	—	—	—	—	—
Extra ribs	2	—	8†	22††	9†	—	—	—	—	—
Skeletal malformations										
Fissura sterni	—	—	—	—	1	—	—	—	—	—
Agnathia	—	—	—	—	2	—	—	—	—	—
Missing vertebrae	—	—	—	2	—	—	—	—	—	—
Brachimelia	—	—	—	—	—	—	—	—	1	—

[a] Including poorly ossified sternebrae, bipartite vertebra centra and shortened 13th ribs.
† $P < 0.05$; †† $P < 0.01$ (Mann Whitney U test).

(Table I). No external malformations were found. Examination for visceral malformations showed dilated urinary tracts, the frequency of which did not exceed that found in the controls, as well as 4 cases of slight internal hydrocephaly all belonging to the same litter and also showing considerable retarded growth (Table II). Retarded skeletal growth was more frequent than in the controls ($P < 0.05$). No skeletal anomalies or malformations were found in this group (Table II).

Another group was exposed to concentrations of 1000 mg/m^3 (266 ppm) of toluene during their entire pregnancy (from day 1 to day 21) for 8 h/day. No maternal mortality occurred in this group, and none of the parameters (maternal weight gain, fetal loss, mean fetal and placental weights and percentage of weight retarded fetuses) differed from the air inhaling controls (Table I).

No external malformations occurred, and the only visceral malformation, dilatation of the urinary tracts, did not differ in frequency from the controls (Table II). There was an increased frequency of signs of retarded skeletal development ($P < 0.05$), but no skeletal anomalies or malformations occurred (Table II).

Xylene

None of the rats inhaling 1000 mg/m^3 (230 ppm) xylene continuously from day 9 to day 14 of pregnancy died. Maternal weight gain was found the same as in the air inhaling controls, there was no increase in fetal loss, and the mean fetal and placental weights as well as the percentage of underweight fetuses did not differ from the controls' respective parameters (Table I). External malformation — agnathia — was found in 2 of the 286 fetuses. Examination for visceral malformations revealed dilated urinary tracts occurring with the same frequency as in the controls (Table II). Signs of retarded skeletal development showed an increasing trend of incidence, but the difference was not significant. As regards skeletal anomalies, the frequency of fused sternebrae and extra ribs increased ($P < 0.05$ for both). In addition to the 2 cases of agnathia mentioned, 1 fetus was found with fissura sterni (Table II).

DISCUSSION

None of the pregnant animals died as a result of benzene inhalation, but their body weight gain was half of the value found in the controls. Some of the pregnant rats inhaling toluene died, — in agreement with the higher doses applied and the higher acute toxicity of toluene [19—21] — the survivors did not, however, differ from the controls in maternal body-weight gain and fetal loss, since toluene does not cause as severe alterations as are caused by benzene in the long run [19—21]. The xylene exposed animals did not differ from the controls in respect of either mortality or body weight gain.

No teratogenic effect of these solvents was found in continuous exposure to 1000 mg/m^3 (313 ppm) benzene or (230 ppm) xylene from day 9 to 14

of pregnancy, to 1500 mg/m^3 (399 ppm) toluene during the same period of pregnancy, or in mice exposed to 500 mg/m^3 (133 ppm) toluene from day 6 to day 13 of pregnancy. None of the solvents increased the incidence of external, visceral or skeletal malformations. All 3 had, however, embryotoxic effect as indicated by retarded fetal development (low fetal weight, signs of retarded skeletal development) and by the increased incidence of skeletal anomalies.

Considerable retardation in fetal development was caused by benzene if applied in the middle-third of gestation, by toluene in the first third, while the retarding effect of toluene was mild in mice exposed in the middle third of gestation and in rats exposed during the entire period of pregnancy for 8 h/day. No retardation was found in rats if the mothers were exposed to toluene and xylene in the middle third of pregnancy.

Each of the 3 solvents caused an increase in the incidence of skeletal anomalies (extra ribs, fused sternebrae) when the pregnant animals were exposed during the middle third of pregnancy.

Several factors are probably jointly responsible for the embryotoxic effects of benzene, toluene and xylene. Having low molecular weights, any of them may pass the placental barrier [22] and act directly on embryonal cells. Toluene e.g., is known to change the permeability of the cell membrane thereby making the passage of various macromolecules into the cell possible [23]. Due to their cardiovascular effects [24,25] the 3 solvents also damage placental circulation. Benzene causes anaemia in the mother and thus further interferences with fetal blood supply. A nutritive effect may also be postulated: the metabolites of toluene and xylene are excreted conjugated to glycine [26,27], and thus the detoxication of the solvents strongly depletes the protein stores of the pregnant mother known to have an increased protein demand and to be extremely susceptible to protein deficiency. For the excretion of benzene metabolites glucuronic acid is necessary, the synthesis of which is a process of high energy demand. Phenol, the metabolite of benzene, inhibits DNA synthesis as shown by Moeschlin and Speck [28] in bone marrow cells in vivo, and by Dobashi [29] in human lymphocytes in vitro. No such effect of xylene has been reported, while toluene has been proved to be free of it [30].

The fact that the animals had been kept in the inhalation chamber had no harmful effect on them, as shown by the air inhaling control group, but stress due to direct irritative effect of the solvents cannot be excluded. Such a possibility is also corroborated by the increased incidence of skeletal anomalies, first of all of extra ribs, which were found to occur in response to treatment with each of the 3 solvents. Although extra ribs cannot be regarded as malformations, an increase in their frequency may indicate stress to the mother or may signalize a teratogenic effect of higher concentrations [31]. The applied doses of toluene were high enough, since further rises would have led to extreme maternal mortality. In the case of benzene and xylene, application of higher doses would be desirable to be able to exclude teratogenicity. Different types of xylene (*o*-, *m*-, *p*-) show different acute

toxicity, and that of their mixture is also different [32], LD_{50} of *o*- and *p*-xylene being, e.g., about two thirds of similar doses of *m*-xylene (Ungváry, pers. comm.) The different types of xylene may also differ in teratogenicity. The absence of teratogenic effect found in the case of xylene mixtures does not mean that the individual types are similarly innocent. This possibility can only be tested by applying pure *o*-, *m*- and *p*-xylene.

Summarizing it may be stated that although none of the solvents have been found teratogenic except for certain skeletal anomalies, they did have embryotoxic effects.

An important practical conclusion that has emerged from the study is that toluene exposure in early pregnancy may cause retardation of fetal development which is not made up for by the end of gestation even if exposure is discontinued.

It is concluded that women in jobs with a risk of benzene, toluene and xylene exposure should be immediately removed from the hazardous environment when pregnancy is established.

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