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Special Report on Ingested Inorganic Arsenic

Skin Cancer; Nutritional Essentiality

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Preface

The U.S. Environmental Protection Agency (EPA) Risk Assessment Forum was established to promote scientific consensus on risk assessment issues and to ensure that this consensus is incorporated into appropriate risk assessment guidance. To accomplish this, the Risk Assessment Forum assembles experts from throughout the EPA in a formal process to study and report on these issues from an Agency-wide perspective.

For major risk assessment activities, the Risk Assessment Forum may establish a Technical Panel to conduct scientific review and analysis. Members are chosen to assure that necessary technical expertise is available. Outside experts may be invited to participate as consultants or, if appropriate, as Technical Panel members.

Major scientific controversies have existed for many years within EPA concerning the health effects of exposure to ingested arsenic. To help resolve these issues, a Technical Panel on Arsenic was formed within EPA by the Risk Assessment Forum. The Technical Panel was charged with preparing a report on arsenic health effects for Agency-wide concurrence and use.

External Peer Review

A draft of this report was reviewed at a peer review workshop of scientific experts in Hunt Valley, Maryland, on December 2-3, 1986. The workshop was highly instructive for the EPA Technical Panel, and the current draft incorporates many of the peer reviewers' comments.

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The Technical Panel acknowledges with appreciation the special contributions of Dr. Vicki Dellarco, Dr. David Jacobson-Kram, Mr. Paul White, Dr. Ken Brown, Dr. Kerrie Boyle, and Ms. Pamela Bassford.

EPA Risk Assessment Forum (1986-87)

Drafts of this report were reviewed by EPA's Risk Assessment Forum in October 1986 and in March 1987. In July 1987, the final report was submitted to EPA's Risk Assessment Council for concurrence.

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Science Advisory Board Review

The Science Advisory Board's (SAB) Environmental Health Committee was asked to review the Risk Assessment Council's science policy statement in the November 1987 draft report that recommended modification of the Risk Assessment Forum's skin cancer risk estimate. The SAB advised the Council that the request was beyond the scope of its activities and was unable to comply.

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I. Overview

Arsenic exposure has long been associated with several different forms of human cancer. The association between inhaled arsenic and an elevated risk of lung cancer is well documented (Enterline and Marsh, 1980; Lubin et al., 1981; Welch et al., 1982; Lee-Feldstein, 1983). Other studies have reported an association between ingested inorganic arsenic and an increased incidence of nonmelanoma skin cancer in a Taiwanese population (Tseng et al., 1968; Tseng, 1977; hereafter "Taiwan study") (Appendix A). Also, exposure to ingested arsenic is associated with an elevated but unquantifiable risk for cancer of internal organs (e.g., liver, kidney) in some studies (Chen et al., 1985, 1986).

The U.S. Environmental Protection Agency's Health Assessment Document (HAD) for Inorganic Arsenic (U.S. EPA, 1984a) contained qualitative and quantitative carcinogen risk assessments for both inhalation and ingestion routes of exposure. Several EPA offices raised questions about the assessment for the ingestion exposure, including: the validity of the Taiwan study and applicability of the dose-response assessment to the U.S. population, the interpretation and use of arsenic-associated skin lesions, and the role of arsenic in human nutrition (the "essentiality" issue).

A Technical Panel was convened by the Risk Assessment Forum to address these issues. In the course of its deliberations, the Technical Panel examined several other issues relating to hazard identification and dose-response assessment for arsenic-induced skin cancer, including some aspects of the pathology of arsenic-associated skin lesions, the genotoxicity of arsenic, the metabolism, body burden, and distribution of this element, and the possibility of threshold effects. The Technical Panel's findings are summarized in the Executive Summary (Part II) and detailed in the remainder of this report. Additional technical analyses appear in the five appendices.

A draft of the Technical Panel's Special Report was peer reviewed at a public workshop held in Hunt Valley, Maryland, on December 2-3, 1986. The Panel revised its report in line with many helpful peer review comments and presented a revised document to the Risk Assessment Forum on March 27, 1987. The Forum's comments and recommendations have been incorporated.

This report is designated as a "Special Report" to distinguish this analysis, which is deliberately limited to the skin cancer and nutritional essentiality issues identified above, from comprehensive risk assessments that fully analyze all indicated health effects and fully conform with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986; hereafter "cancer guidelines"). The Special Report addresses many of the hazard identification,

dose-response assessment (Appendix B), and risk characterization parameters called for in the cancer guidelines, but it does not fully assess or characterize arsenic risks for skin cancer nor does it analyze the other cancers associated with exposure to this element. [1]

Agency scientists and decision-makers should be aware that the lifetime cancer risks and other analyses in this report apply to a form of cancer that is treatable and that generally has a good survival rate in the United States. For this reason, the estimates for arsenic-induced skin cancer may have different implications for human health status than comparable numerical estimates would have for more fatal forms of cancer, including arsenic-induced lung cancer for which the lifetime cancer risk is 4.3×10^{-3} per µg/cubic meter. Because an examination of the regulatory significance of this difference was beyond the purview of the Risk Assessment Forum, the Forum directed this question to EPA's Risk Assessment Council.

The Council's comments and guidance for Agency decisions on arsenic-related skin cancer risk were endorsed by EPA Administrator Lee M. Thomas in a June 21, 1988 memorandum to EPA offices.

Summary

For several years the Agency has debated the issue of the carcinogenicity risk associated with the ingestion of inorganic arsenic. Last year, the Risk Assessment Forum (Forum) completed a reassessment of the problem and issued its finding in a Special Report on Arsenic. The Report, which was extensively peer-reviewed by outside experts, concludes that, based on the scientific data available and in keeping with the Agency's Risk Assessment Guidelines, the cancer potency (slope factor) for human ingestion of inorganic arsenic should be in the range of 3 to 7×10^{-5} $(\mu g/L)^{-1}$. This is a reduction of about one order of magnitude from the estimate generated in 1984 and reflects a more detailed analysis of the available scientific data. To facilitate implementation of the reassessment, I am adopting the Risk Assessment Council's (Council) recommendation that a single value of 5×10^{-5} $(\mu g/L)^{-1}$ be used.

The Council discussed a series of important issues which go beyond the factors considered when EPA quantifies carcinogenic risks. The Council went on to recommend that in making case-specific risk management decisions, program offices should be aware of qualities and uncertainties of a carcinogenic risk estimate for ingested inorganic arsenic that might mitigate

[1]There is evidence of an association between arsenic ingestion and an elevated risk of cancer of various internal organs (e.g., lung, liver, bladder) (see Part III, Section A and Appendix C). This association is not discussed in detail in this report because information needed to quantify the dose-response for internal cancers was not available. As developed in Parts V and VI, the available information merits consideration in the overall assessment of arsenic risk to humans, and further research is warranted.

The *skin cancer* analysis presented here, as well as the ancillary issues discussed in connection with this analysis, supersedes corresponding discussions in the 1984 HAD. The Panel recommends, however, that EPA offices consult the HAD for information on the other forms of arsenic-induced cancer and other arsenic health effects. Also, as explained in the cancer guidelines (U.S. EPA, 1986), appropriate exposure information must be considered along with the health effects data to develop complete risk assessments for this element.

their concerns compared to estimates of risks for other carcinogens. In the Council's view, these qualities and uncertainties could, in a specific risk management situation, modify one's concern downwards as much as an order of magnitude. In such instances, the management document must clearly articulate this fact and state the factors that influenced such a decision.

Background

There is general agreement that inhalation of inorganic arsenic is associated with the development of lung tumors in humans. The available data are adequate to quantitatively estimate the magnitude of the associated risks.

The case of ingestion of inorganic arsenic is more complicated and has been the source of considerable controversy. First, the principal scientific evidence of human carcinogenicity of ingested arsenic is found in a series of epidemiologic studies which were conducted in other countries and whose appropriateness to the assessment of risk to the U.S. population has been called into question. Second, the primary tumor response in these human studies is skin tumors, which are more likely to be detected and successfully treated and much less likely to lead to death than are the lung tumors associated with arsenic exposure via the inhalation route. Third, limited animal evidence suggests that arsenic might be an essential nutrient, although there are no relevant human data at this time. It has been argued that taking action to reduce the level of arsenic below some critical level (as yet unspecified) may reduce any potential cancer risk only at the expense of other decrements to human health.

These difficulties in assessment have led to a range of interpretations and positions in different offices and different Regions. Therefore, the matter was referred to the Risk Assessment Forum for consideration.

Risk Assessment Forum Special Report on Arsenic

The Forum worked diligently to address these issues and others that arose during the deliberations. The considerable efforts of Agency scientists were supplemented by a workshop involving an international panel of experts on the subject, including some of the authors of the principal studies. As a result, the Forum was able to resolve many issues, to the extent permitted by science. Extensive peer reviews, both internal and external, concurred with the conclusions of the Forum.

In summary, the Forum concluded that a series of studies conducted in Taiwan on a large human population that ingested inorganic arsenic in drinking water, together with confirmatory studies in other locations, demonstrates that arsenic is a human carcinogen by the oral route, which puts the chemical in Category A of the Agency's scheme for designating the weight-of-evidence. Further, the Forum concluded that the Taiwan studies provide a reasonable basis for quantitatively assessing the risk of skin cancer associated with the ingestion of inorganic arsenic in this country, despite many uncertainties.

Employing methods in keeping with the Risk Assessment Guidelines, the Forum used the Taiwanese data and estimated the cancer potency (slope of the dose-response curve) to be 3 to 7 x 10^{-5} $(\mu g/L)^{-1}$. This range is roughly an order of magnitude less than the slope factor calculated in 1984. The change primarily reflects modifications in risk assessment methodology and better estimates of the exposures involved in the epidemiology studies used to estimate potency.

The Forum noted that the slope of the dose-response curve may be less than linear and might not pass through the origin. In such a case the calculated slope factor would overestimate the true risk.

The Forum reaffirmed the finding that the skin tumors expected from this exposure would, most often, not result in death. The Forum noted, but did not explore in depth, the existence of data suggesting a link between human

ingestion of inorganic arsenic and the occurrence of internal cancers. Finally, the report concludes that while it is plausible that arsenic is a nutritional requirement in animals and a possible requirement in humans, additional studies are needed to decide the question definitively.

Risk Assessment Council Action

In a series of meetings, the Council discussed the Forum's Special Report, which they found to contain a solid analysis of the science, a clear consensus on the conclusions, and a discussion of the data gaps and associated uncertainties. The Council approved the Report as submitted. The Report represents considerable progress in consolidating a consistent Agency view on the risks of ingested inorganic arsenic, but uncertainties remain which would permit a range of interpretations of the science.

First, the Council believes that, from an implementation point of view, the potency is better expressed as a single value, 5×10^{-5} $(\mu g/L)^{-1}$, rather than a range. This is particularly true in this case where the range is small; i.e., 3 to 7×10^{-5} $(\mu g/L)^{-1}$.

Second, the Council believes that the uncertainties which are currently unresolvable on a scientific basis are best accounted for in the risk management portion of the decision-making process. Specifically, on a case-specific basis, the Council recommends that risk managers reach their judgments in light of the knowledge that:

1. Ingested inorganic arsenic is a class A carcinogen resulting in an increased incidence of skin cancers.
2. Only a fraction of the arsenic-induced skin cancers are fatal.
3. The non-fatal skin cancers remain of some concern.
4. The dose-response curve for the skin cancers may be sublinear, in which case the cancer potency in this Report will overestimate the risks.
5. Arsenic may cause cancer in internal organs.
6. Arsenic is a possible but not proven nutritional requirement in animals. There are no direct data on the essentiality of arsenic in humans.

Conclusion

Based on the Risk Assessment Council's review of the Forum's Report on inorganic arsenic, I am recommending that:

a. Risks of skin cancers associated with the ingestion of inorganic arsenic be estimated using a cancer potency (slope factor) of 5×10^{-5} $(\mu g/L)^{-1}$, derived in the Forum's Special Report.

b. In reaching risk management decisions in a specific situation, risk managers must recognize and consider the qualities and uncertainties of risk estimates. The uncertainties associated with ingested inorganic arsenic are such that estimates could be modified downwards as much as an order of magnitude, relative to risk estimates associated with most other carcinogens. In such instances, the management document must clearly articulate this fact and state the factors that influenced such a decision.

II. Executive Summary

A. Background

A Technical Panel of the U.S. Environmental Protection Agency's Risk Assessment Forum has studied three special issues regarding certain health effects, particularly skin cancer, associated with arsenic ingestion: (1) the validity of the Taiwan study and its use for dose-response assessment in the U.S. population, (2) the interpretation and use of skin lesions reported as arsenic-induced skin cancers, and (3) the role of arsenic as an "essential" nutritional requirement in the human diet. The Technical Panel also reviewed auxiliary information on genotoxicity, metabolism, and other factors that might suggest the most appropriate approach to dose-response assessment.

In brief summary, the analysis shows a causal relationship between ingestion exposure to arsenic and an increased risk of skin cancer. This leads to classification of this element as a Group A human carcinogen under EPA's cancer guidelines (U.S. EPA, 1986). Analyses of data on genotoxicity, metabolism, and pathology yielded information on possible carcinogenic mechanisms for arsenic. However, there is not sufficient information to evaluate a dose-response according to any specific mechanism that one may postulate. In the absence of fully persuasive evidence for any of the possible mechanisms, a generalized multistage model that is linear at low doses was used to place an upper bound on the expected human cancer dose-response.

Using data from a human population for which the lowest dose level in drinking water was approximately 10 μg/kg/day, the maximum likelihood estimate (MLE) of skin cancer risk for a 70-kg person consuming 2 liters of water per day contaminated with 1 μg/L arsenic ranges from 3×10^{-5} (based on Taiwanese females) to 7×10^{-5} (based on Taiwanese males). In other terms, the MLE of risk due to 1 μg/kg/day of arsenic intake ranges from 1×10^{-3} to 2×10^{-3}. These estimates are about an order of magnitude lower than those presented in the 1984 HAD. These risk estimates are based on a dose-response model that assumes linearity at low doses and would overestimate risk if risk decreases faster than linear at low doses or if a threshold for arsenic-induced skin cancer exists.

The available data on nutritional "essentiality" do not fully resolve the questions raised. Arsenic is a possible but not proven nutritional requirement in animals. If arsenic is in fact an essential nutrient in animals, it is likely to be essential in humans, but there are no data on this issue. If arsenic is essential, there is no clear scientific basis for deciding how to use this information in relation to the dose-response information.

This report summarizes the Technical Panel's review and analysis of relevant data. To fully characterize the risk from arsenic exposure in human populations, exposure information and the 1984 HAD on the inhalation route of exposure must be considered along with the findings in this report. A brief synopsis follows.

B. Validity of Data from Taiwan

The Technical Panel believes that results from the Tseng et al. (1968) and Tseng (1977) studies demonstrate a causal association between arsenic ingestion and an elevated risk of skin cancer subject to certain limitations. These investigators studied the prevalence of hyperpigmentation, hyperkeratosis, and skin cancer in 40,421 residents of 37 Taiwan villages in which arsenic in well-water ranged from <0.001 ppm in shallow wells to 1.82 ppm. The 428 cases of skin cancer (10.6/1,000) showed a clear-cut increase in prevalence with exposure. No cases of skin cancer, hyperpigmentation, or hyperkeratosis were reported in a comparison population of 7,500 people who were essentially not exposed to arsenic in drinking water.

Reliance on these data is based on several considerations: (1) the study and comparison populations were large enough (40,421 and 7,500, respectively) to provide reliable estimates of the skin cancer prevalence rates; (2) a statistically significant elevation in skin cancer risk among the exposed population over the comparison population was observed many years after first exposure; (3) the data show a pronounced skin cancer dose-response by exposure level; (4) the exposed and comparison populations were similar in occupational and socioeconomic status, with arsenic-contaminated water the only apparent difference between these two groups; and (5) over 70% of the observed skin cancer cases were pathologically confirmed.

There are also important uncertainties in the studies of the Taiwanese population, including (1) chemicals other than arsenic in the drinking water, which may have confounded the observed association between skin cancer and arsenic ingestion; (2) the lack of blinding of the examiners, which may have led to a differential degree of ascertainment between the exposed and comparison populations; and (3) the role of diet in the skin cancer response observed in the exposed population. The influence of these uncertainties remains to be determined, but they signal a need for cautious characterization of the risk.

Given the findings in this and other studies (see Appendix A), arsenic is classified as a Group A human carcinogen for which there is sufficient evidence from epidemiologic studies to describe a causal association between exposure to this agent and human cancer.

C. Biological Considerations for Dose-Response Assessment

To develop the dose-response assessment, the Technical Panel considered auxilary information on the pathology of arsenic-associated skin lesions, genotoxicity, and the metabolism of this element that might shed light on biological or chemical processes leading to arsenically induced cancer. The Technical Panel looked particularly for information that would help determine whether arsenically induced cancer is more appropriately analyzed using non-threshold or threshold assumptions, and whether arsenic-induced carcinogenicity is linear at low doses.

The Panel studied the possibility that nonmalignant arsenic-induced skin lesions (e.g., hyperpigmentation, hyperkeratosis) occur more frequently at exposure levels below which skin cancer is observed, providing a basis for analyzing arsenic-induced skin cancer as a threshold phenomenon. The Panel found, however, that these lesions are not always precursors to malignant lesions and that some malignant lesions arise *de novo*. Thus, characterization of the skin lesions established end points of interest for dose-response assessment, and suggested that nonmalignant lesions may

serve as useful biological markers of exposure to arsenic, but did not resolve uncertainties regarding nonthreshold approaches for quantifying arsenical skin cancer.

Data from genotoxicity studies raise a number of questions. Arsenic does not appear to induce point mutations, but arsenicals increase the frequency of sister chromatid exchanges and chromosome breakage in cultured cells, including human cells. Such chromosome breaks could lead to stable chromosome aberrations, which require a minimum of two hits with a loss or exchange of genetic material, events that would be compatible with nonlinear kinetics and, therefore, a sublinear dose-response relationship.

Information on the absorption, deposition, and excretion of ingested arsenic shows that arsenic is handled by enzymatic and nonenzymatic reactions. It shows that, except for high exposure levels, inorganic arsenic is converted non-enzymatically to arsenite (+3). *In vivo* methylation of arsenic to monomethyl and dimethyl arsenic (the latter being the major methylated metabolite) appears to be a route of detoxification for acute effects and a general route of elimination. Although some data suggest that methylating capacity in humans can become saturated, studies to delineate the role of biomethylation in chronic arsenic toxicity are needed. Arsenic is known to deposit in certain organs, including the skin, liver, lung, and kidney, a pattern compatible with arsenic-associated cancer in these organs.

Scientists at EPA and elsewhere, faced with uncertainty about mechanisms of chemical carcinogenesis, often analyze chemical carcinogens as though simple genetic changes initiate a carcinogenesis process that is linear at low levels of exposure. Extrapolation procedures from high to low doses then depend on models that are also linear at low doses. Since for arsenicals, as for a number of other carcinogens, there is no evidence of point mutations in standard genetic test systems, the single-hit theory for chemical carcinogenesis may not be applicable. Similarly, the structural chromosomal rearrangements that have been implicated in some cases of carcinogenesis would be expected to require at least two "hits", if not more. In addition, the known toxic effects of the inorganic arsenicals are not inconsistent with the idea that multiple interactions are involved in producing adverse cellular effects.

While consideration of these data on the genotoxicity, metabolism, and pathology of arsenic has provided information on the possible mechanism by which arsenic may produce carcinogenic effects, a more complete understanding of these biological data in relation to carcinogenesis is needed before they can be factored with confidence into the risk assessment process.

D. Dose-Response Assessment

The data from Taiwan have several strengths for quantitative risk assessment: (1) the number of persons in the exposed population and the comparison populations (40,421 and 7,500, respectively) is large; (2) the number of skin cancer cases in the exposed population is relatively large (428 observed); (3) the skin cancer prevalence rates are reported by 12 different age and dose groups; and (4) the data show a pronounced skin cancer dose-response.

At the same time, limitations in the Taiwanese studies introduce uncertainties regarding applicability of this information to the U.S. population. These uncertainties include: (1) the potential exposure to sources of arsenic other than drinking water (e.g., diet) which could result in an overestimation of the cancer risk; (2) the higher case-fatality rate and earlier median age of

onset for Blackfoot disease, which may also be arsenic related, thus resulting in an underestimation of cancer risk; and (3) differences in diets other than arsenic content, between the Taiwanese and U.S. populations, which could modify the carcinogenic response to arsenic observed in Taiwan. (The diet of the arsenic-exposed population was reported to be "low in protein and fat and high in carbohydrates, particularly rice and sweet potatoes.")

Skin cancer cases in these studies included squamous cell carcinoma, basal cell carcinoma, *in situ* squamous cell carcinoma (Bowen's disease), and Type B keratoses, which Yeh (1973) defines as intraepidermal carcinomas. Type A keratoses were defined by Yeh (1973) as benign tumors. Although these keratoses are also found in the exposed population and may pose a carcinogenic hazard, they were not included in the quantitative estimate of cancer risk because of uncertainty regarding their progression to squamous cell or basal cell carcinomas. In addition, there was no information on age-specific prevalence rates for this lesion.

The Technical Panel developed the dose-response assessment using a multistage extrapolation model that incorporates low-dose linearity. This choice was guided by principles laid down by the Office of Science and Technology Policy (OSTP, 1985) and in EPA's cancer guidelines (U.S. EPA, 1986), which set forth the principles that follow.

> No single mathematical procedure is recognized as the most appropriate for low dose extrapolation in carcinogenesis. When relevant biological evidence on mechanism of action exists (e.g., pharmacokinetics, target organ dose), the models or procedures employed should be consistent with the evidence. When data and information are limited, however, and when much uncertainty exists regarding the mechanism of carcinogenic action, models or procedures which incorporate low dose linearity are preferred when compatible with the limited information.

The multistage model chosen by the Technical Panel differed from the model used in the Agency's Health Assessment Document for Inorganic Arsenic (U.S. EPA, 1984) in that the current model is both linear and quadratic in dose. Other changes between the current model and that presented in 1984 include the use of a life-table approach in the current analysis to calculate a lifetime risk of skin cancer. The previous estimate of risk was a lifetime estimate, assuming that an individual lived to be 76.2 years of age. The current model uses a maximum likelihood approach whereas the previous model was a least squares linear regression of prevalence rates. Also, the current analysis assumes that Taiwanese males in the arsenic-endemic area of Taiwan drank 75% more water than does the U.S. population. The current analysis also estimated a risk from the data on Taiwanese females, which was not done in the 1984 analysis and assumed that Taiwanese females drink the same amount of water per day as does the U.S. population.

Based on the current model and the Taiwanese data, the MLE of cancer risk for a 70-kg person who consumes 2 liters of water per day contaminated with 1 μg/L of arsenic ranges from 3×10^{-5} (on the basis of Taiwanese females) to 7×10^{-5} (on the basis of Taiwanese males); or, equivalently, the MLE due to 1 μg/kg/day of arsenic intake from water ranges for 1×10^{-3} to 2×10^{-3}. These estimates are about an order of magnitude less than those presented in the 1984 HAD. Data from two studies (Cebrian et al., 1983; Fierz, 1965) were not suitable for dose-response estimation because of lack of information on population age structure or lack of a control group. These

studies were suitable, however, for comparing with the Taiwanese-based risk estimates, and were consistent with the dose-response for Taiwan.

The proportion of nonmelanoma skin cancer cases in the United States attributable to inorganic arsenic in the diet, the largest arsenic exposure for most Americans, is quite low. Assuming that the dietary intake of inorganic arsenic, including the intake from water and beverages, is 0.25 μg/kg/day and has been constant for the past 85 to 100 years, the number of skin cancer cases per year attributable to inorganic arsenic in food, water, and other beverages would be 1,684. This is about 0.34% of the 500,000 cases of nonmelanoma skin cancer cases that occur among U.S. caucasians each year. For reasons described in the text, even 0.34% is an overestimate, however.

E. Nutritional Essentiality

The Technical Panel also reviewed several studies on arsenic as a possible essential element in the diet to determine the overall impact of arsenic exposure on human health. The information bearing on whether arsenic may be an essential element in human nutrition is incomplete. The studies of chickens and goats suggested that adverse growth and reproductive effects may be attributable to arsenic deficient diets, and that arsenic may be required in the diets of these animals. The Technical Panel is unaware of comparable studies in human populations. While it is plausible that arsenic is a nutritional requirement in animals and a possible requirement in humans, additional studies are needed.

In the absence of definitive information, the likelihood that arsenic is a human nutrient must be weighed qualitatively along with risk assessment information for carcinogenic effects. There is little information to determine the levels of arsenic that would be essential in the human diet, the nature of any human effects, or the degree to which current dietary levels are adequate. It is reasonable to assume, however, that there is no sharp threshold of essentiality and that a spectrum of effects would occur below adequate levels, with the adverse effects of arsenic deficiency *increasing* in severity as exposure is reduced. The risk of cancer would *decrease* as exposure is reduced, but some risk is assumed to exist at all levels of exposure. At low levels of exposure, it is possible that both could occur.

F. Conclusion

The Technical Panel concludes that the Taiwan study demonstrates a causal association between arsenic ingestion and elevated skin cancer risk. In considering the weight of the human evidence of carcinogenicity, the possibility of bias, confounding, or chance has been considered. However, there is a strong dose-response relationship, and independent studies in other countries are concordant in showing the association between arsenic ingestion and elevated skin cancer risk.

Using a multistage model of the skin cancer dose-response data for Taiwan, the MLE of lifetime cancer risk for a 70-kg person who consumes 2 liters of water per day contaminated with 1 μg/L of arsenic ranges from 3×10^{-5} (on the basis of Taiwanese females) to 7×10^{-5} (on the basis of Taiwanese males). The MLE due to 1 μg/kg/day of arsenic intake from water ranges from 1×10^{-3} to 2×10^{-3}. Although the absence of point mutations in genetic tests and certain metabolic information provide some basis for considering alternative risk assessment approaches, conservative assumptions are consistent with arsenic's known carcinogenic effects in

human populations, and an absence of significant information that provides a sound basis for an alternative approach.

An important consideration in evaluating the estimated risks has to do with the nature of the carcinogenic response following arsenic exposure. Basal cell carcinomas generally do not metastasize and, thus, do not have much potential to cause death. They may invade locally, however, and if not attended to, can spread to vital centers and lead to morbidity and death. Squamous cell carcinomas have some potential to metastasize to contiguous structures. Mortality for squamous cell carcinomas is greater than for basal cell carcinomas, but is lower than that for the other primary skin tumors, malignant melanomas (not associated with arsenic exposure).

In summary, skin cancers arise in humans following certain exposures to arsenical compounds. The tumors are generally superficial, easily diagnosed and treated, and are associated with lower mortality than cancers at most other sites. Certain internal cancers also appear to be associated with arsenic exposure. Lacking definitive information on mechanism of carcinogenic action and pharmacokinetics, the Agency has relied on a linear model for extrapolation from higher to lower daily exposures to place an upper bound on the dose-response estimates. Even in the absence of definitive biological information, aspects of the analysis, including lack of genotoxicity and pharmacodynamic considerations, suggest that a linear extrapolation may overestimate the risks from low-level arsenic exposure. Risks may fall off faster than linearly and it is possible that thresholds might exist, but additional data are needed to develop this premise.

III. Hazard Identification and Epidemiologic Studies Suitable for Dose-Response Evaluation

A primary issue before the Technical Panel was the validity of the Taiwan study (Tseng et al., 1968; Tseng, 1977), which had been used in developing the 1984 quantitative risk assessment for skin cancer from ingested arsenic. After reviewing the epidemiologic literature, which includes many reports of an association between arsenic exposure and skin cancer (see Appendix A), the Panel focused on three studies. The Panel found that the Taiwan study provided evidence of a causal association between arsenic ingestion and skin cancer in humans, resulting in its classification as a Group A human carcinogen under EPA's cancer guidelines (U.S. EPA, 1986). Two other studies (Cebrian et al., 1983; Fierz, 1965) showing a skin cancer response from arsenic ingestion were used for comparison with predictions from the dose-response seen in the Taiwan study.

A. Preliminary Considerations

Several of the studies reviewed in this section describe medical conditions other than arsenic-induced skin cancer. Before the epidemiologic studies are discussed, clarification of these conditions are needed.

As discussed below, sun-induced skin cancer features skin lesions comparable in many respects to those produced by arsenic. However, since arsenic-induced skin cancer generally occurs on parts of the body where sun-induced skin cancer lesions are rarely found, the former can be distinguished from the latter.

Blackfoot disease or gangrene is another medical condition observed in areas of chronic arsenicism. In the Taiwan study, persons with Blackfoot disease were more likely to have developed skin cancer than persons who did not have Blackfoot disease. Because Blackfoot disease patients in Taiwan had a low survival rate and because Blackfoot disease had an earlier median age of onset than did skin cancer, it is possible that some potential cancer cases among the Blackfoot disease cohort died without being counted in the Tseng et al. (1968) prevalence study.

Finally, excess incidences of some life-threatening malignancies (e.g., cancer of the lung, liver, and bladder) are observed in arsenic endemic areas. This information has not been fully used in this report because data necessary to quantify risk (e.g., dose-response data, information on mortality rates, and population age structure) were not available to EPA. Studies and case reports that describe an association between arsenic ingestion and internal cancer are briefly reviewed in Appendix C. Additional data from the studies by Chen et al. (1985, 1986) showing an association between internal cancer of several sites and arsenic ingestion have been requested for use in dose-response estimation.

B. Review of Studies

Three studies identified in the literature review are suitable for quantitative evaluation of skin cancer risk. Two are retrospective studies of persons exposed to arsenic in drinking water and one is of persons who had been treated with a trivalent arsenical medicinal (Fowler's solution). As stated above, none of the studies reviewed for this report provides enough data to quantify the internal cancer dose-response due to arsenic ingestion.

1. Taiwan Study

Tseng et al. (1968) and Tseng (1977) reported the results of a large cross-sectional survey concerning health problems of persons living in an area of Taiwan where there were high concentrations of arsenic in the artesian well water supply. Use of these wells began in the years 1900 to 1910. The wells were reported to be 100 to 280 meters deep, with 80% being between 120 and 180 meters in depth. The wells were drilled to solve the problem of drinking water in the area since the water from shallow wells near the seacoast was often salty. Water from the shallow wells was usually free from arsenic (<0.001 ppm), although some had a considerably higher concentrations (1.097 ppm). In 1956, water containing 0.01 ppm arsenic was piped to many places from the reservoir of the Chia-Nan irrigation system. In February 1966, a tap water supply was made available to almost the whole endemic area in Tainan County. (Personal communication with Drs. Tseng and Chien-Jen Chen of the National Taiwan University indicates that the artesian wells are still used [to some extent] during dry periods.) The arsenic level in the wells varied somewhat over time but appeared to be highest during Taiwan's rainy season. In the early 1960s the concentrations of arsenic in the different wells ranged from 0.01 to 1.82 ppm.

By 1965, physical examinations had been performed on a total population of 40,421 in 37 villages. The entire population in all villages in the study area numbered 103,154. The period of the survey was not specified by the authors in their publication, but personal communication indicates that the survey period was about 2 years. Investigators gave special attention to hyperpigmentation, hyperkeratosis, and skin cancer. A control population of 7,500 persons, with age distribution similar to that of the study population but from areas in which arsenic was not endemic in the drinking water supply, was examined in the same way as the arsenic-exposed persons. The arsenic in the drinking water of this comparison population ranged from non-detectable (detection limit not specified) to 0.017 mg/L. Males in the study and control populations were engaged in similar occupations (fishing, farming, and salt production). Four hundred and twenty-eight cases of skin cancer (10.6/1,000) were found in the study population. Of these, 153 were reported to be histologically confirmed. There were no cases in persons less than 20 years old and the prevalence increased markedly with age, except for women over 70. The male-to-female skin cancer prevalence ratio was 2.9:1. There was a clear-cut increase in prevalence with exposure.

Of the 428 people with clinically diagnosed skin cancer, 72% also had hyperkeratosis[2] and 90% had hyperpigmentation. Seventy-four percent of

[2]These are assumed to be benign hyperkeratoses as opposed to the Type"B"hyperkeratoses described by Yeh (1973) as intraepidermal carcinomas and which were counted as skin cancer.

the malignant lesions were on areas not exposed to the sun. Ninety-nine percent of the people with skin cancers had multiple skin cancers. Yeh (1973) studied 303 of the 428 skin lesions originally reported by Tseng et al. (1968) histologically: 57 were squamous cell carcinomas; 45 were basal cell carcinomas (28 deep, 17 superficial); 176 were intraepidermal carcinomas (23 Type B keratoses, 153 Bowen's disease); and 25 were combined forms.

The prevalence rate for Blackfoot disease was 8.9 per 1,000 in the study population. Prevalence rates for keratosis and hyperpigmentation in the study population were 183.5 and 71 per 1,000, respectively. The youngest patient with hyperpigmentation was 3 years old, the youngest with keratosis was 4, and the youngest with skin cancer was 24.

No cases of skin cancer, Blackfoot disease, hyperkeratosis, or hyperpigmentation were found in the control population of 7,500. One could argue that this suggests a potential bias on the part of the examiners since they were not "blinded" as to whether the persons being examined were from the arsenic area or not. Thus, they might have made a greater effort to ascertain cases in the study population than in the comparison population. All of the study subjects were examined by the same physicians according to a common protocol however, the disease was relatively easy to diagnose differentially (Chen et al., 1986). Furthermore, over 70% of the skin cancer in the exposed population were histopathologically confirmed. Lastly, at least with regard to skin cancer, the fact that no cases were found in the comparison population is not inconceivable, since the expected number of skin cancer cases in the control population of 7,500 persons (using the skin cancer rate for Singapore Chinese from 1968 through 1977) is a little less than 3. Using this as the expected prevalence, the probability of observing no cancer cases is 0.07.

Subsequent analysis of the drinking water revealed substances other than arsenic including bacteria and ergot alkaloids (Andelman and Barnett, 1983). Neither of these two substances has been previously associated with skin cancer, and it seems unlikely that these two substances could be considered confounders. Also, as outlined in Appendix A, a multitude of studies have demonstrated an association between arsenic ingestion and skin cancer. It seems unlikely that the same confounders that might have been present in the Tseng et al. (1968) study would have been present in the other studies as well. Chen noted, however, that the presence of substances in the well water other than arsenic, although not confounding, might have produced a synergistic effect (Chen, 1987).

2. *Mexican Study*

Cebrian et al. (1983) and Albores et al. (1979) reported the results of a prevalence study of individuals living in two towns in the Region Lagunera section of Mexico (the exposed town of El Salvador de Arriba and the control town of San Jose del Vinedo Diego). The two towns are 37 km apart, are very similar with regard to economic and atmospheric conditions, and have similar age and sex distributions except for the over 60 age groups where the proportion of individuals was slightly greater in the control town. The only apparent important difference between them is in the level of exposure to arsenic in water. Monitoring from August 1975 to May 1978 showed the average arsenic level to be 0.411 $\pm$ 0.114 mg/L (20 samples) in El Salvador de Arriba and 0.005 $\pm$ 0.007 mg/L (18 samples) in San Jose del Vinedo Diego (in each case about 70% pentavalent, 30% trivalent), varying somewhat over time. Historical exposure levels are not known; organoarsenical pesticide

runoff into the water supply may have been an additional source of arsenic (in both towns) before 1945.

Dr. Mariano Cebrian (1987), the primary investigator, indicates that there was one well per community, and that the well was located in the center of each of the respective towns. Each well had been drilled to a depth of about 70 to 100 meters. The water was then distributed to approximately ten holding tanks from which the residents drew their water. In addition to arsenic, fluoride was also reported to be present in the water supply of the exposed town. Arsenic concentrations in the water supply were reported to correlate with fluoride concentrations in the Region Lagunera (Cebrian, 1987). Chemical analysis was not done for any substances other than fluoride and arsenic.

Every third household in the two towns was sampled, and each member present in the household was examined. Data on exposure sources and number of years of exposure were obtained by means of questionnaires from 296 people from El Salvador de Arriba and 318 people from San Jose del Vinedo Diego. Physical examinations were performed on each resident in the sampled households to assess hyperpigmentation, hypopigmentation, papular and palmoplantar keratoses, and ulcerative lesions.

A 3.6-fold greater risk of ulcerative lesions, compatible with a clinical diagnosis of epidermoid or basal cell carcinoma, was reported in the exposed population as compared to the controls. This report was based on four cases (which were not histologically confirmed) from El Salvador de Arriba (prevalence rate of 14/1,000) and no cases from San Jose del Vinedo Diego. In contrast to the observation of Tseng et al. (1968), there was no sex difference in the distribution of lesions. The shortest latency period for skin cancer (one case) was 38 years which was also the age of the individual (age was similar to residence in 75% of the patients.) Of the remaining three cases, two were in the 50 to 59 age group and one was in the $\geq$ 60 age group. Hypopigmentation was discovered in 17.6% of the exposed persons, hyperpigmentation in 12.2%, and palmoplantar keratoses in 11.2%. No biopsies were taken. No other skin lesions were reported for the exposed town; however, peripheral vascular disease such as that reported in Taiwan (i.e., Blackfoot disease) has also been reported in the arsenic endemic area of Region Lagunera in Mexico (Salcedo et al., 1984).[3] The shortest latency for hypopigmentation was estimated to be 8 years, for hyperpigmentation and palmoplantar keratosis 12 years, and for papular keratosis 25 years. Based on average drinking water arsenic concentrations of 0.41 mg/L, Cebrian calculated the following minimum total ingested doses for the development of cutaneous toxicity: hypopigmentation, 2 g; hyperpigmentation, 3 g; keratoses, 3 g; invasive carcinoma, 2 g. The minimum detection time and the lowest cumulative dose may have been overestimated, since it is not known at what age the lesions may have first become clinically apparent. A few classical arsenic-induced skin lesions were identified in the control population: hypopigmentation in 2.2%, hyperpigmentation in 1.9%, and palmoplantar keratosis in 0.3% (Cebrian et al., 1983). The authors speculated that the occurrence of lesions in the control town may have resulted from ingestion of foodstuffs produced in the same region and contaminated with arsenic.

[3]The reported Blackfoot disease in Mexico and Taiwan is consistent with a report (Borgono and Greiber, 1972) of Blackfoot disease in an area of Chile where there is arsenic contamination of the water supply.

In contrast to the situation in Taiwan, the Mexican population had limited water supplies, thus enabling more accurate estimates of exposure. This study also presents potential problems, however. The study may be biased since the examiners knew who were exposed and who were not. The possibility of preferential diagnosis may not have been as great in this study as it was in the Taiwan study, since cutaneous signs other than ulcerative lesions were observed in the control population. Also, there was no estimate of non-response (i.e., the number of individuals not present at the time of the interview and/or examination is not reported).

3. *German Study*

Fierz (1965) reported on a retrospective study of patients treated with a 1:1 dilution of Fowler's solution containing 3.8 g arsenic/L. An accurate assessment of the total arsenic intake was available from patient records. A total of 1,450 patients were identified as having received arsenic treatment 6 to 26 years previously. Invitations for a free medical examination were mailed to them. Two hundred sixty-two persons presented themselves for examination; 100 patients refused to participate, and 280 could not be located. The status of the other 808 persons to whom invitations had been mailed was not reported. Of the 262 examined, 64 had been treated with Fowler's solution for psoriasis, 62 for neurodermatitis, 72 for chronic eczema, and 64 for other disease. Twenty-one cases of skin cancer were found, comprising 8% of the subjects examined. Multiple carcinomas were found in 13 of the 21 patients; 10 of these were multiple basal cell carcinomas, described as polycyclic, sharply bounded erythemas with slight infiltration. Single basal cell carcinoma, squamous cell carcinoma, and Bowen's disease were less frequently encountered. Of the 21 patients with carcinomas, 16 showed distinctly developed "arsenic warts" on the palms and soles, simultaneously with skin tumors. The author estimated the minimum and mean latency period for carcinomas to be 6 and 14 years, respectively. However, the latency period did not appear to be correlated with dose.

Hyperkeratosis was the most frequent sign of arsenic toxicity, occurring in 106 of 262 (40.4%) of the patients. In patients who had received the equivalent of 3 g of arsenic as the diluted Fowler's solution, the incidence of hyperkeratosis was 50%. The minimal latency period for hyperkeratosis was reported to be 2.5 years; the mean latency period was not reported. Melanotic hyperpigmentation was found in only 5 of 262 persons (2%); however, 3 persons reported that they had looked "stained" shortly after taking arsenic, but that this condition had regressed over the years. The incidence rates of both skin cancer and hyperkeratosis increased with dose. The size of the hyperkeratoses also increased with dose. The author also found that the original diagnosis (psoriasis, neurodermatitis, chronic eczema, or acne) did not affect the development of skin cancer when dose was controlled for.

One problem with this study is that a significant proportion of the exposed population did not participate in the study. Three hundred and eighty persons of a total of 1,450 (59%) refused to participate or could not be contacted. It is not known what became of 808 other persons to whom invitations had been mailed. The author classified the 262 who did present themselves for examination into three groups: those satisfied with the results of the arsenic treatment and wishing to express thanks; those in whom side effects were occurring (e.g., skin cancer, hyperkeratosis, etc.); and those who were still suffering from the initial disease and who were eager to get consultation. This description makes apparent the possibility of selection bias. Another problem is the lack of a control group.

C. Summary

The Taiwan (Tseng et al., 1968; Tseng, 1977), Mexican (Cebrian et al., 1983), and German (Fierz, 1965) studies have been discussed in detail because they have been used as part of the dose-response assessment in Part V. Additional reports of the association of arsenic ingestion and cancer risk are found in Appendix A. (Reports of an association between ingested arsenic and cancers of internal organs are discussed in Appendix C.)

Strengths of the Taiwan study include: (1) the study and comparison populations were large enough (40,421 and 7,500 respectively) to provide reliable estimates of the skin cancer prevalence rates, (2) a statistically significant elevation in the skin cancer prevalence among the exposed population over that of the comparison population was observed many years after first exposure, (3) there was a pronounced skin cancer response by arsenic exposure level, (4) the exposed and comparison populations were similar in socioeconomic status and occupation with the only apparent difference between the two populations being that of arsenic exposure, and (5) over 70% of the observed skin cancer cases were pathologically confirmed.

Important uncertainties of the Taiwan study include: (1) chemicals other than arsenic in drinking water which may have confounded the observed association between skin cancer and arsenic ingestion, and (2) the lack of blinding of the examiners which may have led to a differential degree of ascertainment between the exposed and comparison populations. Another uncertainty relates to the possibility that diet may have modified the response.

The Mexican study found the prevalence of skin cancer increased in a population exposed to arsenic via drinking water versus a comparison population, but the sample sizes of the exposed and comparison groups (296 and 318, respectively) were much smaller than the Taiwan study. Futhermore, there were only four cases of skin cancer among the exposed. The German study of patients who ingested arsenical medicinals reported a skin cancer dose-response by the amount of arsenic ingested, but there was no comparison group and many of the exposed population did not participate in the study. Both studies (Mexican and German), despite their limitations, were considered useful for quantitative comparison with the results from Taiwan. (See Part V. Dose-Response Estimate for Arsenic Ingestion)

In reviewing the weight of the human evidence of carcinogenicity, the possibility of bias, confounding or chance has been considered. However, there is a strong dose-response relationship, and independent studies in other countries are concordant in showing the association between arsenic ingestion and elevated skin cancer risk.

Considering the above, arsenic is classified as a Group A human carcinogen (U.S. EPA, 1986), for which there is sufficient evidence from epidemiologic studies to support a causal association between exposure to this agent and cancer.

IV. Selected Elements of Hazard Identification

This part summarizes biological information relating to the skin cancer dose-response for ingested arsenic. Section A reviews certain pathologic features of skin lesions associated with arsenic exposure and comments on their significance. Section B summarizes the genotoxicity of arsenic and discusses its role in the cancer dose-response assessment. Section C highlights relevant metabolic information.

A. Pathologic Characteristics and Significance of Arsenic-Induced Skin Lesions[4]

Several aspects of arsenical skin lesions are briefly reviewed here to provide a background for distinguishing the nature and relative health impact of the skin lesions upon which the dose-response assessment is based. The discussion also shows that certain lesions may serve as biological markers of early arsenic exposure. Subsection 1 describes the pathology of the various skin lesions; subsection 2 discusses the interrelationship between these lesions with respect to progression from a preneoplastic stage to a malignant neoplasm; and subsection 3 examines the case-fatality rate of basal cell and squamous cell carcinoma.

1. Description and Malignant Potential of Skin Lesions

Several different skin lesions that are described in various reports of arsenic-exposed humans are discussed. Yeh et al. (1968), in his study of patients with chronic arsenicism, provides the most complete description of the various skin lesions, particularly hyperpigmentation, hyperkeratosis, and skin cancer. Skin cancer, as defined by Yeh et al. (1968), includes intraepidermal carcinomas (Type B keratosis and Bowen's disease), basal cell carcinomas, invasive squamous cell carcinomas, and "combined lesions."

Hyperpigmentation is a pathologic hallmark of chronic arsenic exposure and may occur anywhere on the body, typically as dark brown patches showing scattered pale spots. Hyperpigmentation is not considered to be a malignant neoplasm or a precursor to malignancy. Although it may occur together with hyperkeratosis, hyperpigmentation does not appear to be directly related to hyperkeratosis (i.e., they are *not* different stages in the evolution of a single type of lesion, but, rather, are of different cellular lineage and are related only because of their common cause).

[4]An expert pathologist, Dr. D. S. Strayer of the University of Texas Medical School at Houston, was asked by the EPA Risk Assessment Forum to review the literature on arsenical skin pathology. Subsections 1 and 2 of this section are based on that review.

Yeh et al. (1968) and Yeh (1973) reported that arsenical hyperkeratosis occurs most frequently on the palms of the hands and soles of the feet; however, hyperkeratosis may occur at other sites. Hyperkeratoses usually appear as small corn-like elevations, 0.4 to 1 cm in diameter. Yeh (1973) concluded that in the majority of cases, arsenical keratoses showed very little cellular atypia and are morphologically benign. Thus, Yeh (1973) divided the arsenical keratoses in the Tseng study[5] (1977; Tseng et al., 1968) into two groups: Type A, which included mildly atypical cells, and a malignant Type B, which included cells with more marked atypia. Authors of some other studies do not make this distinction. Yeh et al. (1968) stated that keratotic lesions of chronic arsenicism, although histopathologically similar, were distinguishable from Bowen's disease. Some pathologists, however, state that arsenical keratoses are difficult to distinguish from Bowen's disease; some considered them one and the same (Hugo and Conway, 1967). As discussed later, Type B keratoses may evolve into invasive squamous cell carcinoma.

Bowen's disease, an *in situ* squamous cell carcinoma, represents a continuation of the dysmaturation processes observed in Type B keratoses. These lesions may become invasive, but the frequency is not known. These lesions are sharply demarcated round or irregular plaques that may vary in size from I mm to more than 10 cm, and tend to enlarge progressively. Arsenic-associated Bowen's disease is usually multifocal and randomly distributed and the lesions tend to arise on the trunk more often than do arsenical hyperkeratoses.

Arsenical basal cell carcinomas most frequently arise from normal tissue, are almost always multiple, and frequently occur on the trunk. The superficial spreading lesions are red, scaly, and atrophic and frequently indistinguishable from Bowen's disease by clinical examination.

Arsenical invasive squamous cell carcinomas (referred to as epidermoid carcinomas in Yeh (1973) and Yeh et al. (1968) arise from normal tissue or within preexisting hyperkeratoses or Bowen's disease. Persistent fissuring, erosion, ulceration, and induration are key clinical features. Although arsenic-associated squamous cell carcinomas do not differ histopathologically from sun-induced squamous cell carcinomas, they can be distinguished by their common occurrence on the extremities (especially palms and soles) and trunk; sun-induced squamous cell carcinomas appear primarily on sun-exposed areas (i.e., the head and neck).

Finally, several reports describe "combined lesions" that were considered attributable to arsenic that include both basal cell carcinomas and Bowen's disease (Yeh et al., 1968), or mixed squamous cell carcinomas and basal cell carcinomas (Sommers and McManus, 1953). Whether these represent true mixed lesions or coalescence of two separate lesions has been debated by Sanderson (1976). He argues that because arsenical skin cancer includes multiple foci, separate foci of the same type of neoplasia or two different types of adjacent neoplasias may eventually collide and blend together, producing a "combined lesion."

In summary, distinguishing characteristics of lesions of arsenical skin cancer, include multiplicity and distribution on unexposed parts of the body (e.g., palms of the hands, soles of the feet, other parts of the extremities, and

[5]The Tseng study is the epidemiologic study that forms the basis of the cancer risk estimate associated with ingested arsenic (see Sections B and C).

trunk). Sun-induced basal cell carcinomas do not metastasize and the metastatic potential of squamous cell carcinomas is low; whether this is also true for arsenical skin cancer is unknown. As discussed in subsection 3 of this section, there is some basis for speculating that arsenical skin cancer may have a higher metastatic potential than sun-induced skin cancer.

2. *Progression of Skin Lesions*

The interrelationship between the various lesions of chronic arsenicism was examined to further characterize lesions that would be used to develop the dose-response assessment. For example, the frequency of transformation from the benign lesions to the malignant lesions would better characterize the proportion of benign lesions that might be factored into the dose-response assessment.[6] Progression of lesions was also examined to provide a qualitative discussion of carcinogenic mechanisms that might indicate the suitability of a particular extrapolation model. There was not enough information on progression of lesions in arsenic-exposed humans for the Technical Panel to develop a mechanistic model. As suggested in section C of this part, future studies may provide useful information.

The development of arsenical keratosis and Bowen's disease into invasive squamous cell carcinoma is documented in certain instances (see Table 1). Note in Table 1 that Yeh et al. (1968) also cited one basal cell carcinoma that arose from keratotic lesions. Whether the keratoses referred to in the table are of type A or B as described by Yeh et al. (1968) is unknown. The frequency of malignant transformation, however, is difficult to determine because many case reports of arsenical skin cancer do not specify the pre-existing condition of the skin. Moreover, analysis of some reports is complicated by lack of histopathologic examination or by uncertain terminology.

Invasive squamous cell carcinoma, basal cell carcinoma, and Bowen's disease ("*in situ*" squamous cell carcinoma) were used as end points for the cancer dose-response assessment. Type B keratoses were also included since Yeh et al. (1968) had classified them as an intraepidermal carcinoma which, by inference, were malignant. Although the Type A keratoses were classified by Yeh et al. (1968) as benign, they may have malignant potential. Type A keratoses were not used in the dose-response assessment, however, because there was a lack of information on the distribution of Type A keratotic lesions by age and dose, and the malignant potential was not clearly established. Hyperpigmentation was not included in the dose-response assessment since hyperpigmentation is not a malignant condition, and it does not appear to be a pre-malignant stage in nonmelanoma skin cancer. Both of these lesions are indicators of arsenic exposure, and can serve as biological markers.

3. *Case-Fatality Rate of Arsenic-Induced Skin Cancer*

The Technical Panel examined the public health impacts of arsenic-induced skin cancer for U.S. residents by using case-fatality rates for skin

[6]The EPA cancer guidelines (U.S. EPA, 1986) state that "Benign tumors should generally be combined with malignant tumors for risk estimates unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same histogenic origin."

Table 1. Invasive Malignant Transformation of *In Situ* Arsenic-Induced Skin Lesions

Author, year, MOE	Total number of patients	Total number with keratoses	Malignant Transformation From: Ker	From: BD	To: SCC	To: BCC	To: NS	Number of Malignant *de novo* or NS
Hutchinson, 1888, med	5	5	5	0	-	-	5	0
Geyer, 1898, water	37	35	2	0	-	-	2	0
Montgomery,[a] 1935, med	87	85	3	1	4	0	0	1
Arguello, 1938, water	39	39	10	0	9	0	1	29
Prunes, 1946	14	14	13	0	0	0	13	0
Neubauer 1947, med[b]	137	116-133	30	0	10	1	19	107
Sommers, 1953, med	5	5	1	1	2	0	0	3
Roth, 1957, occ	27	NS	5	0	4	0	1	NS
Graham and Helwig, 1963, med	15	15	1	0	1	0	0	2
Fierz, 1965, med	262	106	1	0	1	0	0	20
Yeh, 1968, 1973, water	40,421	2,868	24	?20[c]	24 ?20	0	0	384
Zaldivar, 1974, water	120 adults	most	2	0	2	0	0	0
	337 children	NS	0	0	0	0	0	0

[a]Cited by Zaldivar, 1974.
[b]Not including cases reported by Hutchinson (1888) and Montgomery (1935).
[c]Yeh indicated that 20 probably arose from Bowen's disease.

MOE = method of exposure; Ker = keratoses; BD = Bowen's disease;
SCC = squamous cell carcinoma; BCC = basal cell carcinoma;
NS = not specified; med = medicinal; occ = occupational.

Source: Shannon and Strayer, 1987.

cancer, data that give the cumulative incidence of death among people who develop this condition. However, since data on case-fatality rates for arsenic-induced skin cancer in the United States are not available, the Technical Panel drew on two sources to estimate the case-fatality rate of arsenic-induced skin cancer in the United States. The most direct information upon which to estimate a case-fatality rate from arsenic-induced skin cancer in the United States would be derived from U.S. arsenic-exposed populations. However, the only case-fatality rate reported for an arsenic-exposed population is that of Yeh (1973), who observed a 5-year case-fatality rate of 14.7% for patients with arsenic-induced skin cancer in Taiwan.

Differences in medical care between the Taiwanese and U.S. populations may lead to different case-fatality rates in the two countries. Thus, approximations of the case-fatality rates for basal and squamous cell carcinoma for both males and females in caucasian U.S. populations were derived from aggregate data on nonmelanoma skin cancer and are presented in Table 2; these data primarily reflect sun-induced skin cancer. Table 2 shows that nonmelanoma skin cancer, which is the most common malignant neoplasm among caucasians in the United States (Scotto and Fraumeni, 1982), is rarely fatal; less than 2% of all nonmelanoma skin cancer cases die from the disease. These low case-fatality rates probably reflect the ease of diagnosis and effectiveness of treatment. Case-fatality rates could not be calculated for nonwhites due to lack of data on nonmelanoma skin cancer incidence rates.

In conclusion, the estimated case-fatality rate attributable to arsenic-induced skin cancer ranges between <1% (U.S. populations) to 14.7% (Taiwanese populations). There is currently not enough information to determine whether the case-fatality rates in Table 2 or that based on the Yeh data realistically describe the probability of death in the United States due to arsenic-induced skin cancer. The higher case-fatality rate of 14.7% reported by Yeh may reflect differences in medical treatment between Taiwan and the United States or may reflect differences in disease aggressiveness for arsenic exposure relative to sun exposure resulting from several factors. For example, arsenical nonmelanoma skin cancer often appears as multiple lesions on the body, presenting a higher probability of metastasis. Arsenic-induced skin cancer has a higher squamous to basal cell ratio than does nonmelanoma skin cancer in the United States, the majority of which, as stated above, is believed to be sun-induced, and squamous cell carcinoma has a higher probability of metastasis than does basal cell. Finally, arsenic-induced skin cancer tends to occur on the trunk and extremities, areas that are not generally sun-exposed. Lesions in these areas may not be as readily detected by the patient or physician, thus increasing the probability of not diagnosing the disease until a more advanced stage.

B. Genotoxicity [7]

1. Introduction

Various inorganic compounds of arsenic have been tested for mutagenicity in a variety of test systems ranging in complexity from bacteria to peripheral

[7]With permission of the authors, this discussion is adapted from a review article prepared by Jacobson-Kram and Montalbano (1985) and the U.S. EPA Health Assessment Document for Inorganic Arsenic (U.S. EPA, 1984a).

Table 2. **Estimated Case-Fatality Rates for Nonmelanoma Skin Cancer by Cell Type[a]**

Race-sex group	Cell type	Incidence rate/ 100,000[a]	Estimated Mortality rate/ 100,000[b]	Estimated case-fatality rate[c]
White male	Squamous cell	65.5	0.8	1.2%
White male	Basal cell	202.1	0.2	<0.1%
White female	Squamous cell	21.8	0.3	1.4%
White female	Basal cell	115.8	0.08	<0.1%

[a]Based on annual incidence rates, age-adjusted to the 1970 U.S. population (Scotto and Fraumeni, 1982).

[b]Race-specific nonmelanoma skin cancer mortality rates were obtained from Riggan et al. (1983) and are age-adjusted to the 1970 U.S. population. An assumption, based on Scotto and Fraumeni (1982) was made for this analysis that squamous cell carcinoma deaths accounted for 80% of the race-sex specific age-adjusted mortality rate.

[c]Estimated case-fatality rate = Estimated mortality rate/incidence rate (MacMahon and Pugh, 1970). The following three assumptions were made: (1) incidence of nonmelanoma skin cancer remains stable for a period corresponding to the longest duration of the disease in the individual; (2) the distribution of disease duration remains stable; and (3) the proportion of patients with various outcomes (death or recovery) remains stable. All assumptions are believed to be met since disease duration is relatively short and survival is good.

lymphocytes of exposed human beings. Although much of the data presents many questions, the weight of evidence leads to five conclusions:

(1) Arsenic is either inactive or extremely weak for the induction of gene mutations *in vitro*.

(2) Arsenic is clastogenic and induces sister chromatid exchanges (SCE) in a variety of cell types, including human cells, *in vitro*; trivalent arsenic is approximately an order of magnitude more potent than pentavalent arsenic.

(3) Arsenic does not appear to induce chromosome aberrations *in vivo* in experimental animals.

(4) Several studies suggest that human beings exposed to arsenic demonstrate higher frequencies of SCE and chromosomal aberrations in peripheral lymphocytes.

(5) Arsenic may affect DNA by the inhibition of DNA repair processes or by its occasional substitution for phosphorous in the DNA backbone.

Several reviews on the mutagenicity of arsenic are available (Jacobson-Kram and Montalbano, 1985; Flessel, 1978; National Academy of Sciences, 1977; Leonard and Lauwerys, 1980; World Health Organization, 1981).

2. *Possible Mechanisms of Genotoxicity*

Arsenic is unusual in several respects. First, unlike the majority of clastogenic agents, arsenic does not appear to directly damage DNA except, perhaps, at highly cytotoxic doses. Rather, it seems to have its effect through some interference with DNA synthesis. This contention is supported by observations that arsenic induces chromosomal aberrations and SCE only when it is present during DNA replication. Incubation and removal of arsenic before DNA synthesis has no effect (Nordenson et al., 1981; Crossen, 1983).

Second, arsenic is unusual in that it induces chromosomal aberrations and SCE while it fails to induce gene mutations. In this regard it is like benzene, another unusual carcinogen (Dean, 1978). Although capable of producing chromosome aberrations as well as gene mutations, x-irradiation is much more potent for the former end point. There is a small possibility, however, that the discrepancy for arsenic is an artifact. Protocols for gene mutation assays generally involve cellular incubation with the test agent for relatively short time periods (2-3 hr), while protocols for aberrations often involve the presence of the test agent for one or two entire cell cycles (12-48 hr). Thus, in the latter protocol, arsenic would be present for at least an entire S-phase for all cells, whereas, when tested for gene mutations, arsenic would be present for only a small fraction of the S-phase in approximately one-third to one-half of the cells. Since the evidence available suggests that arsenic has its effect only during DNA replication, this may account for the discrepancy.

Arsenic has long been known to be a sulfhydryl reagent capable of inhibiting a number of thiol-dependent enzyme systems, trivalent forms being much more potent than pentavalent forms (Leonard and Lauwerys, 1980). Thus, one possible mechanism of action for arsenic would be the inhibition of DNA repair enzymes. The work of Rossman in bacteria (1981) and Jung et al. (1969) in human cells *in vitro* lend support to this hypothesis. Also the observations of Sram (1976) on the interactions of arsenic with tris(1-aziridinyl) phosphine sulphide (TEPA) for the induction of chromosomal aberrations and dominant lethals support such a contention. The potencies of trivalent and pentavalent arsenicals as sulfhydryl reagents are similar to their potencies as clastogens and SCE-inducing agents. Observations that counter this hypothesis are the reports by Rossman that arsenic has no effect on the frequency of UV-induced mutations in mammalian cells *in vitro* and that arsenic does not affect the frequency of EMS-induced aberrations *in vivo* (Poma et al., 1981).

Another possible mechanism for the action of arsenic may be through its occasional incorporation into the DNA backbone in place of phosphorous. There are several lines of evidence to support this mechanism. First, for this to occur, arsenic would have to be present during DNA synthesis and would have no effect on nondividing cells. Second, such a mechanism could explain why arsenic is clastogenic (such a bond would be weaker than the normal phosphodiester bond) but does not induce gene mutation. Third, arsenic has been shown to cause strand breaks in DNA (Fornace and Little, 1979). Also, x-irradiation, a potent clastogen and poor inducer of gene mutations, predominantly causes strand breaks as its major DNA lesion. An argument against such a mechanism is the observation that the trivalent forms are more potent than pentavalent forms, while pentavalent arsenic should be more likely to substitute for phosphorous in DNA. Furthermore, arsenic would have to be capable of being phosphorylated.

3. *The Use of Arsenic Genotoxicity Data in the Evaluation of Carcinogenic Risk*

Genotoxicity at low doses is an important indicator of irreversible change in genetic function. Such changes are a critical feature of many postulated mechanisms for chemical carcinogenesis and the basis for ascribing low-dose linearity to carcinogenic processes. Although the lack of genotoxic response does not preclude linearity at low doses, it is potentially important as a consideration in selecting a model for extrapolation of carcinogenic risk.

The *in vitro* dose-response function for the induction of chromosomal aberrations by both trivalent and pentavalent arsenic is linear. It is important to note, however, that most chromosomal aberrations scored in a standard cytogenetics assay, such as that used in the evaluation of arsenic, are lethal events. The cells scored in these assays carry lesions that do not permit them to survive more than one or two additional cell cycles after damage and are, therefore, genetically of no consequence.

Agents that are capable of breaking chromosomes are also capable of causing stable chromosome rearrangements, such as translocations or inversions. To induce such a rearrangement, at least two chromosomes per cell must be damaged (or one chromosome damaged twice). Based on simple target theory, one would expect a nonlinear dose-response relationship for the induction of rearrangements at low doses. In this case, there are two targets per cell, both of which must be hit in order to bring about a rearrangement. At low doses, both targets must be hit in order to bring about a rearrangement, and the possibility of hitting both targets in a single cell is small, but finite. Further, if as discussed above, arsenic acts by interfering with DNA synthesis and repair processes, rather than by causing mutations, the need for two events is compounded by the need for arsenic also to produce toxic effects on DNA synthesizing enzymes. With increasing doses, many cells will contain a single hit and the dose effect curve becomes linear.

The size of any apparent "practical threshold" will be determined by the "size" of the target; i.e., if a high percentage of arsenic molecules interact with chromosomes to cause breaks, the targets are large, and the observed threshold is small. Although these observations suggest the existence of a "practical threshold," there is a measurable "spontaneous" frequency of chromosomal breaks. Because a cell may already carry one break, the induction of the second break (and the resulting rearrangement) would be a single hit phenomenon. Indeed, the induction of dicentrics (a two-hit chromosomal rearrangement) is linear for ionizing radiation even at very low doses. Clearly, these arguments do not support the existence of a threshold, a dose level below which aberrations would not occur. However, the possibility of a nonlinear dose-response relationship at low doses should be recognized.

How chromosomal rearrangements would influence the carcinogenic process is only speculative at this time. Although there are examples of oncogene activation associated with cancers in humans and experimental systems, arsenic-induced chromosomal changes have not been observed *in vivo*, and no data are yet available for arsenic-induced cancers in regard to oncogene activation. While lack of mutagenic activity may argue against the notion that single arsenic-cell interactions may start a process leading to malignancy, gene mutation may not be the only factor leading to low-dose linear dose-response relationships.

C. *Metabolism and Distribution (See Appendix E)*

Inorganic arsenic is a potent poison resulting in adverse effects following acute exposure. Acute toxicity studies indicate that inorganic compounds are more potent than organic forms, and valence state-3 inorganic arsenicals are more toxic than valence state-5 compounds across a number of species. Since the mammalian body can interconvert inorganic arsenic species and can methylate valence state-3 compounds, it appears that methylation is a

means of detoxifying inorganic forms. As more methyl groups are added, the compounds become less and less acutely toxic.

Although there are many data gaps in our understanding of the body's handling of arsenic, great strides have been made in recent years in the ability to speciate among valence states of arsenic. The picture that unfolds is as follows. Inorganic arsenic (+5) can be interconverted in the blood with (+3) - inorganic forms, and the latter can be singularly methylated to form mono-methyl arsenic (MMA); these are enzymatic and nonenzymatic processes. It appears that arsenite, but not arsenate can enter liver cells (at least *in vitro*) where a second methyl group can be added: MMA becomes dimethyl arsenic (DMA) via a rate-limiting enzymatic process.

Under low-level exposures to arsenic, there seems to be a balance between the amount entering the body and the amount being excreted. Most absorbed arsenic is lost from the body in the urine as inorganic arsenite, MMA, DMA, and other, yet uncharacterized, organic forms. A small amount of arsenic is lost by desquamation of the skin.

With increasing arsenic intake there is suggestive evidence that there is some maximal amount the body can readily handle. An early study (Valentine et al., 1979) noted that ingested arsenic in blood did not change as a function of dose until water concentrations exceeded about 100 µg/L. Buchet et al. (1981, 1982) suggest that the body's ability to form DMA seems hampered at exposures in excess of about 500 µg/day, without affecting the excretion of inorganic arsenic or MMA in the urine. If this is the case, then total urinary excretion of arsenic may be compromised at high doses leading to increased tissue levels.

Given the predilection of arsenic for tissues with high sulfhydryl groups, like skin, it seems plausible that high arsenic loads may be associated with increased deposition in the skin. The nature of the binding of arsenic to the skin is unknown at this time; however, radioisotopically labeled inorganic arsenic is retained for longer times than are organic arsenicals. In addition, more drastic chemical treatments are required to remove arsenic from the skin following administration of inorganic than organic arsenic. These pieces of evidence suggest that the binding in the skin after inorganic arsenical exposures is more tenacious and more stable than that following exposure to organic compounds. Although these findings are interesting, the way that they may influence the carcinogenic process, either qualitatively or quantitatively, has not been ascertained.

Another finding is that the methylating capacity of the body may change as a function of exposure, such that maximal levels of excretion of methylated arsenicals are reached after weeks of exposure to the compound. In a like manner, the ability to excrete methylated arsenicals seems to be lost as a function of time after removal of arsenical exposure. Thus, with alternating arsenical intake, individuals may go through periods of efficient metabolism and excretion as well as a tendency to accumulate body stores of arsenic.

It is possible that differences in diet between the United States and Taiwan may have modified the carcinogenic effects of arsenic. The Taiwan diet was reported to be "low in protein and fat; carbohydrates, rice, and sweet potatoes constitute the main part of the diet " (Tseng et al., 1968). It is possible that the reduced protein in the Taiwan diet may compromise the body's ability to methylate and excrete arsenic. Experiments in animals indicate that under methioninedeficient conditions, the body's ability to methylate (Shivapurkar and Poirier, 1983) and excrete arsenic is compromised (Marafante and Vahter, 1986). Some studies in South America where diets seem to be protein adequate, however, indicate that skin cancer still occurs even when the level

of arsenic in the drinking water is about equal to that in Taiwan. Another consideration with regard to diet is that the low fat diets in Taiwan may have had a protective effect against cancer. Boutwell (1983) found that underfeeding animals in fat or calories diminished the cancer occurrence during the promotion stage of skin cancer.

In summary, the metabolism and distribution data are important for evaluating the carcinogenic properties of arsenic. If the interconversion of inorganic arsenic to its methylated forms is saturable, then total urinary excretion of arsenic may be compromised at higher doses, leading to increased tissue levels. The available studies, however, do not contain sufficient information for full evaluation of this hypothesis. In addition, the studies do not identify drinking water exposure levels for humans at which this process may be saturated. Thus, their influence on the carcinogenic process, either qualitatively or quantitatively, is uncertain, but merits further study.

V. Dose-Response Estimate for Arsenic Ingestion

A. Introduction

Dose-response assessment develops a numerical expression for the interrelationship between exposure and carcinogenic response at expected human exposure levels. Because this assessment often includes extrapolation from high doses used in animal studies to low doses in the region of human exposure and from animals to man, consideration of possible mechanisms of cancer development are important in deciding on the most appropriate extrapolation procedures for any particular chemical agent. For ingested arsenic, the dose-response estimate is based on human data (Tseng et al., 1968; Tseng, 1977) for which the lowest dose level was about 10 μg/kg/day.

Low-dose risk estimates based on customary linear assumptions would be overestimates if a threshold exists, or if risk decreases faster than linear as dose decreases. To study these questions, data on genotoxicity, pathology, metabolism, and pharmacokinetics were evaluated, particularly to help determine whether a nonthreshold or a threshold approach was more appropriate for this agent. Because the mechanism by which arsenic induces skin cancer in humans remains unknown and for other reasons developed below, the Technical Panel used a generalized multistage model with a time factor to develop dose-response information on the relationship between exposure to arsenicals and skin cancer in humans.

1. Considerations Affecting Model Selection

After evaluating several factors that might aid in selecting an extrapolation model for cancer risk, the available evidence is not persuasive as to any particular approach, and certain considerations seem to point in different directions. Some considerations suggest that a conservative approach--e.g., methods assuming that there is no threshold for carcinogenic response--is necessary to adequately predict arsenic risks for humans, while others suggest that nonthreshold assumptions will overestimate the risk to humans.

For example, in deciding between nonthreshold and threshold approaches to the dose-response for arsenic, the development of skin lesions in persons exposed to arsenic was evaluated. Nonmalignant lesions (e.g., hyperpigmentation, hyperkeratoses), which are often observed before any indications of malignancy and more frequently than cancer, can serve as biological markers of exposure to arsenic. It is not clear whether these lesions can also be regarded as precursors to cancer that would identify an exposure threshold or level below which exposure to arsenic does not elicit a carcinogenic response. In particular, hyperpigmentation does not appear to progress to cancer, and data are not available on the progression of lesions that Yeh et al. (1968) called Type A hyperkeratosis. Although many squamous cell carinomas arise within pre-existing lesions, most basal cell carcinomas arise *de novo*. This means that Type A hyperkeratoses as a group cannot be viewed as precursors to all skin cancers. Thus, although the possibility of

using data on lesions to identify a threshold for arsenic-induced carcinogenesis is intriguing, additional information is needed before these observations could justify using threshold rather than nonthreshold assumptions.

Other considerations suggest that a less conservative approach is appropriate. Since arsenicals do not appear to induce point mutations, one rationale for assuming low-dose linearity and using the generalized multistage model might not apply, and alternative, less conservative models should be considered. In this regard, structural chromosomal rearrangements that have been implicated in some cases of carcinogenesis could be expected to involve at least two "hits" and may imply a "theoretical" threshold. While such a "threshold" for cancer cannot be proven, any requirement for multiple "hits" would suggest a curvilinear dose-response relationship. Also, pharmacokinetic studies suggesting that tissue dosimetry of arsenic may change dramatically above some yet undisclosed exposure level suggest a nonlinear approach based on nonlinearity of dose. The role of tissue deposition in inducing carcinogenesis is not known but, consistent with dose-response theory, at higher target-organ doses greater biological effects would be expected.

On balance, then, there is a paucity of information on the mechanism of carcinogenic action or the pharmacokinetics of arsenic that leads to confidence that any particular extrapolation approach is more appropriate than another. In these circumstances, it seems reasonable to use an extrapolation model with low-dose linearity to place an upper bound on the expected human cancer dose-response. It is considered an upper-bound estimate because the existing data on arsenic suggest that multiple hit or threshold considerations might apply to the extent these factors influence the carcinogenic process. Thus, in interpreting the risk estimate derived from the linear extrapolation, it is important to keep in mind the possibility that the model overestimates the dose-response to an unknown extent. Certainly, at least some high level exposures are associated with human carcinogenic risk, but as one decreases exposure, risks may fall off faster than linearity. The risk at low doses may be much lower than the current estimates, as low as zero, due to such factors as the metabolism or pharmacokinetics of arsenic.

2. *Changes in Methodology Relative to the 1984 Assessment*

In 1984, EPA estimated the unit risk for arsenic concentrations in drinking water using the data of Tseng et al. (Tseng et al., 1968; Tseng, 1977). Some modifications and additional considerations to the 1984 assessment are made in the current document to calculate a new risk estimate. These modifications include an adjustment for the larger amount of water believed to be consumed by the Taiwanese males in the study population as compared to persons in the United States. The previous estimate assumed that males and females in Taiwan and the United States drink 2 liters of water per day. The current estimate assumes that the Taiwanese male in the study population drinks 75% more water than does a person in the United States. The current assumption is based on the fact that the males of the study population performed heavy outdoor work in a very hot climate. As with the 1984 analysis, the current analysis assumes that Taiwanese females consume the same amount of water per day as a person in the United States (2 liters per day).

Also, the current analysis uses a life-table approach using age-specific U.S. mortality data to calculate a lifetime risk of skin cancers from chronic

ingestion of water containing 1 µg/L of inorganic arsenic. The previous analysis produces an estimate of the risk of developing skin cancer from chronic ingestion of water containing 1 µg/L of inorganic arsenic by age 76.2 years, assuming that one lived to that age. In addition, the current analysis uses a maximum likelihood approach, whereas the previous analysis used a least-squares linear regression of the prevalence rates. The maximum likelihood approach is considered a better approach because it takes account of the relatively small populations in the older age groups. Furthermore, the current analysis used both quadratic and linear dose terms, whereas the previous model was only linear in dose. The fit of the data to the model employing linear and quadratic terms is significantly better than if only a linear term is used ($p < 0.05$).

The cancer risk estimate so derived is then used to predict the number of skin cancer cases that would occur in two other study populations exposed to arsenic via ingestion (Cebrian et al., 1983; Fierz, 1965) for comparison with the number that were actually observed in these studies. The details of these calculations are presented in Appendix B.

B. Estimation of Risk

1. Estimation of Risk Using Taiwan Data

The study by Tseng et al. (1968) and Tseng (1977) (see Part III) provides the best available data for quantitative risk assessment. This study is useful for risk assessment for several reasons. First, it is a study of human populations, a point with obvious advantages for assessment of risk to humans. The exposed and comparison populations were large (40,491 and 7,500, respectively), and prevalence rates in the exposed population were presented according to ages and levels of water concentration so that it is possible to estimate cumulative cancer incidence by age and dose level. The Technical Panel concluded that this study provides an adequate basis for quantitative risk assessment despite the important uncertainties. Of the three studies, it provides the largest study population, ascertained a large number of skin cancer cases, and reported responses by 12 dose and age groups.

The quantitative assessment of hazard for arsenic ingestion uses the generalized multistage model with both linear and quadratic dose assumptions. These calculations show that for the U.S. population, the risk of developing skin cancer from lifetime exposure of 1 µg/kg/day ranges from 1×10^{-3} to 2×10^{-3} (see Table B-4 in Appendix B). Had Singapore skin cancer rates been used to calculate the background cancer rate for the Taiwanese population, the risk estimates are almost the same (see Table B-5). As in previous EPA risk assessments, including the 1984 arsenic risk assessment, the point estimate, rather than the 95% upper bound, is used when human data and a dose-response model with a linear term are used in the calculation. One reason for using the point estimate with human but not animal studies, is that human data usually involve exposure levels that are closer to the exposure range to which one wishes to extrapolate. Secondly, the difference between point and upper-bound estimates is of no practical significance when there is low-dose linearity. Assuming low-dose linearity holds for the Taiwan population, this is especially true for arsenic data because of the large population in that study.

2. Comparison with Mexican Data

Cebrian et al. (1983) (also described in Part III), conducted a prevalence study of skin lesions in two rural Mexican towns, one with arsenic-

contaminated drinking water. The data from this study are not as useful for quantitative risk estimation as those from the Taiwan study because there was only one dose group among the arsenic-exposed persons, and the study populations were relatively small (the exposed and comparison populations numbered 296 and 318, respectively). Moreover, this study identified only four cases of skin cancer. It is useful, however, to compare the dose-responses from the Taiwan study with those in the Mexican population studied. The generalized multistage model developed using the Taiwan data was used to predict prevalence rates for the Mexican population studied by Cebrian.

These calculations show that the model developed from the Taiwan data provides a prediction of skin cancer risk that is consistent with the results of the Mexican study.

3. *Comparison with German Data*

The study by Fierz (1965) (Part III) was, like the Cebrian et al. (1983) study, not as suitable for quantitative risk estimation as the Taiwan study. The poor response rate of the potential study participants, the lack of a comparison group, and the lack of information on dosing patterns were the primary reasons why this study was not used for quantitative risk calculations. However, the results of this study, like those of Cebrian et al. (1983), were compared with estimates of prevalence derived from the Taiwan study.

At the lowest dose in Taiwan (10.8 μg/kg/day), the prevalence rate of skin cancer was 2%. At the equivalent dose in the Fierz study, the prevalence rate of skin cancer is estimated as 3.4% to 15.4%. This 3.4% to 15.4% range is the result of the non-response among the potential study subjects described in Part II, Section A. Further explanation may be found in Appendix B. The Fierz data are not inconsistent with the prevalence of cancer estimated from the Taiwan data. Differences in skin cancer prevalence rates of these two study populations could be due to factors such as the following: the difference in exposure regimens and medium (Fowler's solution is a mixture of potassium arsenite, potassium bicarbonate, alcohol, and water); the difference in the valence states of arsenic (potassium arsenite is trivalent arsenic, whereas the arsenic in the Taiwan wells was mostly pentavalent); other chemicals present; genetic differences among Taiwanese, Mexicans, and Germans (caucasians could be more susceptible); and cultural or socioeconomic conditions.

C. Summary of Dose-Response Evaluation

1. *Numerical Estimates*

Dose-response analysis for skin cancer resulting from exposure to arsenic in drinking water was performed on data from the epidemiologic study conducted in Taiwan. A generalized multistage model in time and dose was used for this analysis. The results were compared to data obtained from epidemiologic studies conducted in Mexico and Germany. These comparisons are not inconsistent with the risk estimates calculated from the Taiwan data.

Based on the Taiwan data (Tseng et al., 1968; Tseng, 1977), the maximum likelihood estimate of lifetime risk of skin cancer for a 70-kg person who consumes 2 liters of water contaminated with 1 μg/L of arsenic per day is calculated to range from 3×10^{-5} (on the basis of Taiwanese females) to 7×10^{-5} (on the basis of Taiwanese males); or, equivalently, the lifetime risk due to 1 μg/kg/day of arsenic intake from water ranges from 1×10^{-3} to 2×10^{-3}.

The skin cancer risk in the United States is unlikely to be greater than these estimates.

2. *Uncertainties*

As described above, qualitative uncertainties in the hazard identification include the possibility of competing mortality from Blackfoot disease, confounding by other chemicals, and lack of blinding of the investigators. In addition, the Technical Panel attempted to quantify two uncertainties in the dose-response evaluation: use of the Taiwan prevalence rate to estimate the cumulative incidence rate, and the influence of arsenic from sources other than drinking water on the Taiwan skin cancer prevalence.

Regarding use of the prevalence rate, one assumption (see Appendix B) in using such data to estimate cumulative incidence rate is that the mortality rates are the same in diseased (skin cancer) and non-diseased individuals. As indicated previously, the arsenic-exposed population in Taiwan had an elevated risk of Blackfoot disease which has an earlier age of onset and a higher case-fatality rate than skin cancer. Also, persons with Blackfoot disease had a higher probability of having skin cancer than persons who did not have Blackfoot disease. This association of skin cancer and Blackfoot disease would have underestimated the risk of skin cancer due to arsenic since some of the persons with skin cancer and Blackfoot disease may have died before being observed in the Tseng et al. prevalence study. The Technical Panel made certain presumptions with respect to differential mortality and estimated its effects on the age-specific skin cancer incidence (see Appendix B). Based on this analysis, the Technical Panel estimated that differential mortality would underestimate the dose-response by no more than 50%.

A countervailing uncertainty relates to arsenic intake by the Taiwan population. Since arsenic-contaminated water was used for vegetable growing and fish farming, food consumption could have been an important source of arsenic in the Taiwan population in addition to the water used for drinking. Not enough information is available on the arsenic content in food, however, for use in the risk calculation. Considering only arsenic in food contributed by water used for cooking, the dose-response may have been overestimated by 30% (see Appendix B).

Finally, absent animal data or reliable human data under conditions of low exposure, the shape of the dose-response, if any, at low doses is uncertain.

3. *U.S. Populations*

To evaluate the contribution of arsenic exposure to the incidence of skin cancer in the United States, the Technical Panel considered estimating the number of cancer cases resulting from inorganic arsenic in the diet. The amount of inorganic arsenic in the diet, including drinking water and beverages, is between 17 and 18 µg/day (see Appendix E). The midpoint of this range, 17.5 µg/day, is equivalent to 0.250 µg/kg/day. Assuming that the amount of dietary inorganic arsenic has remained constant over the past 85 to

100 years (the longest expected lifetime), the annual number of skin cancer cases in the United States resulting from dietary inorganic arsenic would be 1,684 cases per year, based on the data for Taiwanese males (see Table B-4, Appendix B). [8]

In a telephone conversation with Herman Gibb of the Carcinogen Assessment Group (May 1987), Dr. Joseph Scotto of the National Cancer Institute estimates that currently about 500,000 caucasians in the United States develop invasive nonmelanoma skin cancer each year.[9] Thus, the proportion of nonmelanoma skin cancer cases in the United States attributable to inorganic arsenic in the diet, the largest source of arsenic exposure for most Americans, is quite low (0.34%). [10]

Even 0.34% is an overestimate for several reasons. First, the estimate of arsenically induced skin cancer for diet and drinking water is based on skin cancer prevalence data from the Taiwan study which includes both invasive and *in situ* carcinomas. Only 42% of 303 cases that were histopathologically examined in the Taiwan study were invasive nonmelanoma skin cancer cases; the balance (58%) were intraepidermal carcinomas. The estimated annual number of United States caucasian nonmelanoma skin cancer cases cited above as 500,000 includes only invasive nonmelanoma skin cancer. Second, the Taiwan study involved clinical examination of individuals, while the estimate of 500,000 cases in the U.S. population was based on a review of clinical records. Ascertainment of cases will be better by actual examination than by a review of records where cases may not be recorded, all sources of records not examined, or sources of records which are examined are not available or lost. Third, the above estimates of arsenic-induced skin cancer in the United States resulting from arsenic present in the diet and drinking water is based only on the male data from Taiwan. The female data for Taiwan would give an estimate that is more than twofold lower.

Finally, because of socioeconomic and ethnic differences between the United States and Taiwan, the Technical Panel's draft report to the workshop stated that the applicability of these estimates to the U.S. population is of concern. Several workshop participants responded to this stated concern by noting that the United States was a culturally diverse society, as well as a society which included persons of all socioeconomic levels; thus, extrapolation from the Taiwan study to the United States was reasonable.

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[8]This is based on a July 1, 1986, estimate of a U.S. population of 241,596,000 people and the age distribution of the population at that point in time (U.S. Bureau of the Census, 1987).

[9]Not enough information is available for races other than caucasian with which to make reasonable estimates of annual nonmelanoma skin cancer cases.

[10]Although the denominator for this percentage is only caucasian Americans, caucasians constitute 85% of the U.S. population (U.S. Bureau of the Census 1987). Furthermore, the incidence of nonmelanoma skin cancer among nonwhites is considerably less than that of whites (Scotto et al., 1983) so that the number of nonmelanoma skin cancer cases occurring each year among nonwhites is minimal in comparison to the 500,000 cases occurring among whites.

VI. Arsenic as an Essential Nutrient

A. Background

In 1983, the National Academy of Sciences reported that arsenic is an "essential" nutrient for humans.

> Research should also be designed to evaluate the possible essentiality of arsenic for humans--a requirement that has been demonstrated in four mammalian species. In the absence of new data, the conclusion reached in the third volume of *Drinking Water and Health* remains valid, i.e., if 0.05 mg/kg of dietary (total) arsenic is also a nutritionally desirable level for people, then the adequate human diet should provide a daily intake of approximately 25 to 50 µg. The current American diet does not meet this presumed requirement (National Academy of Sciences, 1983).

A report prepared for EPA also concluded that arsenic is essential to human nutrition (O'Connor and Campbell, 1985), and EPA has relied on this assessment in a rule-making action (U.S. EPA, 1985).

In the draft Forum report submitted for peer review, the Technical Panel questioned this conclusion and the role that a nutritional requirement would have in risk assessment for cancer. At the December Peer Review Workshop, the Subcommittee on Essentiality summarized its conclusions on this question as follows:

> (1) Information from experimental studies with rats, chicks, minipigs, and goats demonstrates the *plausibility*[11] that arsenic, at least in inorganic form, is an essential nutrient. A mechanism of action has not been identified and, as with other elements, is required to establish fully arsenic essentiality.
>
> (2) The nutritional essentiality of inorganic arsenic for humans is not established. However, the history of trace element nutrition shows that, if essentiality of an element for animals is established, it is highly probable that humans also require the element. Accordingly, knowing a mechanism of action is needed for a full interpretation of the currently available animal data.
>
> (3) The group consensus position is that, at this time, it is only possible to make a general approximation of amounts of arsenic that may have nutritional significance for humans.

[11] Emphasis added. The term "plausibility" refers to the term as employed in the framework described in Section B, subsection 2, of this part.

(4) Elucidation of the role of arsenic in human nutrition will depend upon development of specific information in the following areas:

- biochemical and physiological mechanisms of action,
- biological activity and metabolic response to various chemical
- species of ingested arsenic, and
- dose-response relationships between animal species.

The scientific data on which these conclusions were based are summarized below,along with some concluding comments on the use of this information in the risk assessment process.

B. Animal Studies

1. Data Summary

Two laboratories have independently reported that arsenic is an essential nutrient in goats and minipigs (Anke et al., 1976; 1978) and in rats and chicks (Uthus et al., 1983).

In a two-generation study, Anke et al. (1976, 1978) compared goats and minipigs that were fed diets containing less than 50 ng arsenic/g (low arsenic) with control animals on diets supplemented with 350 ng arsenic/g.[12] The diet was based on beet sugar and potato starch, with arsenic added to the supplemented diet as arsenic trioxide. There was no effect on the growth of the parental generation (F_0) animals. However, animals fed low-arsenic diets showed depressed fertility; only 58% of the goats and 62% of the minipigs conceived, as compared to 92% and 100% of controls, respectively. The offspring showed depressed birth weights (87% relative to the controls), depressed skeletal ash, and elevated perinatal mortality. Some of the low-arsenic lactating goats died; histological examination revealed ultra structural changes in the myocardium (Schmidt et al., 1984).

Nielsen and coworkers studied the essentiality of arsenic in rats and chicks (Uthus et al., 1983). In the rat study, low-arsenic Sprague-Dawley dams were fed a diet containing 30 ng/g arsenic from day 3 of gestation. Controls received 4.5 µg arsenic (4.0 µg as sodium arsenate, the pentavalent form)/g and 0.5 µg as sodium arsenite. Following weaning, the growth of low-arsenic offspring was slower than that of the arsenic-supplemented controls. The low-arsenic rats appeared less thrifty than controls and their coats were rougher and yellowish. Elevated erythrocyte osmotic fragility, elevated spleen iron, and splenomegaly were noted in these animals.

In a separate three-generation study, dams were placed on a diet that contained less than 15 ng arsenic/g within 2 days of breeding. Controls received a supplement of 2 µg arsenic/g diet, as sodium arsenate. Growth depression was the most consistent effect of the low-arsenic diet observed throughout all three generations (F_1, F_2, and F_3). In a replicate of this study (Uthus et al., 1983), only 2 of 12 low-arsenic F_1 females became pregnant

[12]Although investigators in this field often describe diets as arsenic "deficient" and the animals as arsenic "deprived," since dietary arsenic levels are generally not established, the term "low-arsenic" is used here. Similarly, in most studies, the control animals were maintained on a diet supplemented with arsenic, rather than a standard commercial diet. For this reason, this report uses the term "supplemented" animals or diets.

compared to 9 of 12 controls, and the number of pups per litter was smaller in the low-arsenic group.

In chicks, reduced arsenic (20 ng arsenic/g in the diet) depressed growth after 17 to 20 days (Uthus et al., 1983). In addition, these chicks had larger, darker livers, elevated zinc in the liver[13], elevated erythrocyte osmotic fragility, depressed alkaline phosphatase, and depressed white cell count, as compared to chicks on the supplemented diet. Some dose-effect information may be gleaned from these studies. In the course of these investigations, the arsenic content of the skim-milk powder base varied from 25 ng/g to 45 ng/g. The most marked changes were found in animals ingesting the 25 ng/g diet. The chicks fed 45 ng arsenic/g did not differ from controls, indicating that this may be a minimum requirement for chicks. The presence or concentration of arsenic in the tissues of these animals was not reported.

In an attempt to establish a biochemical function for inorganic arsenic, Nielsen and coworkers have shown nutritional interrelationships in studies using arsenic, zinc, and arginine (Uthus et al., 1983). Similarly, Cornatzer et al. (1983) have studied the role of arsenic in the biosynthesis of phosphatidyl choline (PC). They observed decreased PC biosynthesis in liver endoplasmic reticulum of Sprague-Dawley rats fed a diet containing 14 ng arsenate/g diet as compared with the values observed in rats maintained on a diet supplemented with 2 ppm (2 µg) arsenate/g diet. The authors hypothesized that the observed depression was not caused by a direct effect of arsenic on the enzyme system responsible for PC biosynthesis, but may have resulted from altered amino acid and/or protein metabolism. None of the studies to date have established a biochemical function for arsenic.

Organic forms of arsenic enhance growth in poultry. The concentrations used to enhance growth are at least 500-fold greater than the levels used in the essentiality work. However, organic arsenic is less bioavailable. Thus, in these studies, the effective levels of inorganic arsenic may be comparable to those used in studies of essentiality. Many nutritionists feel that organic arsenic enhances growth in poultry by cleansing the intestinal gut of flora, an antibiotic action. Further work with animals whose guts have been sterilized would be useful in order to confirm this mechanism of growth enhancement and may be useful for interpreting the data on arsenic essentiality.

2. *Evaluation of Data*

The December Workshop's Subcommittee on Essentiality referred to a historical framework for the determination of nutritional requirements.

Data pertinent to application of this framework were described previously in this report. Several laboratory studies described significant differences between animals maintained on low-arsenic diets relative to those on diets supplemented with this element. However, several factors limit the usefulness of these observations.

Information on the composition and adequacy of the basal diets is particularly important in determining the specificity of the deficiencies

[13]The significance of elevated zinc in the liver is not known.

Framework for Determination of Nutritional Essentiality

Empirical Observations	- Establish Plausibility of Animal Models
Reproducible Syndrome	- Use of Chemically Defined Diets, Animal Models
Biochemical Lesions	- Characterize *Specificity* of Lesions
Specific Biochemical Functions *Absolutely* Dependent on Factor	
Essentiality	

observed. For example, Uthus and Nielsen (1985) state that the baseline arsenic diet in their studies was borderline adequate in sulfur amino acids. Furthermore, because details of the diet preparation are not provided in Anke's arsenic reports, the Technical Panel could not assess whether methods used to remove arsenic also destroyed other essential nutrients in the treated food.[14] Factors such as these make it difficult to evaluate fully the role of arsenic deficiency in the reported change in health status.

Despite these limitations, the Technical Panel and Peer Review Workshop participants concluded that these studies provide sufficient information to suggest that a requirement for arsenic in animal diets is plausible, as contemplated in the first step of the framework. However, the available studies provide insufficient information to establish the remaining elements in the framework, i.e., "reproducible syndrome," "biochemical lesion," and "specific biochemical functions dependent on the factor."[15] Since the last two factors are particularly important, the essentiality of arsenic has not been rigorously established, even for animals.

C. Applicability to Humans

The Subcommittee on Essentiality cautioned (see point 3 of their conclusions stated above, and Appendix D) that definition of the requirement for arsenic in human nutrition must await the establishment of its essentiality. They agreed that an order of magnitude estimate is possible. They cautioned, however, that uncertainties influence such an estimate. Among these the reviewers cited lack of knowledge of a biochemical mechanism and

[14]Certain procedures, such as acid washing of corn, were described; chelating agents were not used in preparation of the feed. (Dr. Anke was invited to the December workshop, but was unable to attend.)

[15]As explained in Appendix D, the written report of the Workshop Subcommittee on Essentiality is somewhat incomplete and ambiguous on the current status of steps 2 and 3 in the framework, and the recollections of different workshop participants differ. Some believe that the group concluded that reproducibility (step 2) has been established by the animal data, while others believe that only plausibility (step 1) has been established. The individual comments presented in Appendix D suggest that there was a range of views among the reviewers and, perhaps, that the group was silent on step 2 in the written report because full agreement was lacking.

physiologic role, lack of knowledge of arsenic species in foods, lack of information on the validity of biological species comparison, and inability to specify how a putative intake requirement varies with developmental stage.

Dose-effect information is lacking in the animal studies, which generally compare reduced-arsenic diets to the same diets with substantial supplemental arsenic (for example, 30 ng/g versus 4 µg/g). Despite that lack of information on arsenic levels in animal tissues or food intake that would allow estimates of arsenic doses, several methods have been used for quantitative extrapolations to estimate a human requirement. These methods described below, are highly speculative. Nielsen and coworkers cautiously estimated a human requirement of 30 to 40 µg/day based on the apparent adequacy for chicks of the diet containing 45 ng/g arsenic (Uthus et al., 1983). This estimate assumes that the same intake would be adequate for chicks and humans and that humans consume 700 to 1,000 g of food per day. In other papers, Nielsen estimated human requirements in another way. He assumed a dietary requirement for these animals could be somewhere between 6.25 and 12.5 µg/1,000 kcal. If humans and chicks consume calories in the same way, humans eating 2,000 kcal/day would require 13 to 25 µg daily.

These two estimates are consistent with procedures used by nutritionists to estimate human requirements based on animal data. A method of extrapolation consistent with that used by toxicologists doing risk assessments for toxic effects would use information on the body burdens of animals consuming arsenic-adequate diets, and extrapolating from these data what a human would need to consume to achieve a similar body burden. For example, Nielsen's chicks required 40 ng arsenic/g diet. Assuming that they weighed 0.40 kg and ate 50 g of food per day, they would consume 5 µg arsenic/kg/day. Hove (1938) concluded that 2 µg per day was adequate for a rat; this amount also extrapolates to a dose of 5 µg arsenic/kg/day. If humans have a similar requirement, a 70-kg person would need about 350 µg arsenic/day, almost 10 times the current estimated adult intake. Since it does not appear that current arsenic intake produces arsenic deficiency, this procedure does not seem appropriate for nutritional extrapolation. An extrapolation based on surface area rather than body weight results in an estimate of 24 to 30 µg arsenic/day, which is more nearly consistent with the results of other methods. The estimates should therefore be interpreted as delineating a possible human nutritional requirement of the order of several tens of µg/day.

The Technical Panel is not aware of case reports describing an arsenic requirement for humans, nor of experimental or epidemiologic-type studies designed to determine whether arsenic is essential. Furthermore, if arsenic is a required nutrient for humans, current environmental arsenic exposures are not known to produce human arsenic deficiency.[16] O'Connor and Campbell

[16]Even a well-controlled animal environment appears to provide enough arsenic to confound essentiality studies. In all of the studies of low-arsenic diets, special steps were taken to exclude extraneous arsenic from the animals' environment. For example, goats were kept in polystyrene sties and supplied with cellulose litter. Frequently, more than one generation of low-arsenic exposures was required to produce effects attributed to arsenic deficiency.

(1985) noted that the Food and Drug Administration (FDA) Market Basket Surveys reported a decrease in arsenic (total dietary) from 68 to 21 µg arsenic/day between 1967 and 1974. The FDA has revised its total diet study and is currently reporting higher levels of dietary arsenic, which now may be fairly stable at approximately 46 µg arsenic/day (an unknown fraction is inorganic). Since most estimates of a human nutritional requirement for arsenic fall between 10 and 30 µg/day, the current estimated intake appears to be adequate.

D. Summary and Conclusions

Two groups of investigators have studied the essentiality of arsenic in control animals on conception rate, abortion rate, birth weight, growth, and life expectancy. The results of experiments in the chick and rat are less definitive. The diet used in the latter series of studies varied somewhat in arsenic content, rendering replication difficult, and necessitating use of an artificial diet which may have been borderline deficient in sulfur-containing amino acids.

Despite some limitations in the available literature, the Technical Panel and the workshop participants concluded that the first step in the framework for essentiality has been established, that is, information from experimental studies with rats, chicks, minipigs, and goats demonstrates the plausibility that arsenic, at least in inorganic form, is an essential nutrient.

With respect to the second step, identification of a reproducible syndrome, both the Panel and the workshop peer reviewers concluded that there is insufficient published information available to determine the reproducibility of the arsenic deficiency syndrome. Moreover, the framework outlined above does not require that this be unambiguously shown if a biochemical lesion is demonstrable. A mechanism of action has not been identified and, as with other elements, is required to fully establish arsenic essentiality. The evidence to date does not allow one to identify a physiological role for arsenic.

In sum, the nutritional essentiality of inorganic arsenic for animals has not been established, but is a plausible assumption. If an element is required in animals, it is highly probable that humans also require it. Therefore, although no studies in humans on this question are known to the Technical Panel, a human requirement for arsenic is also possible.

If arsenic were an essential element, one still does not know how to use that information in an assessment of cancer dose-response. One can say that the risks from arsenic deficiency would increase as a function of reductions in exposure below the threshold of essentiality. One might say that cancer dose-response decreases to the threshold for essentiality, but it does not follow that the cancer risk is zero at that point. It is possible that, at doses below an essentiality threshold, the overall risk to an individual would depend on both the cancer and deficiency-induced effects.

VII. Future Research Directions

The significant information gaps identified in this report suggest future research directions relating to cancer risk assessment of ingested arsenic. Crucial gaps in the data base are found for (1) epidemiology, (2) mechanisms of arsenic-induced skin cancer, (3) metabolic phenomena involving arsenic in various species and its impact on the dose-response, and (4) essentiality. Much of the proposed research requires international cooperation. In addition, efforts among different parts of government and the private sector should be integrated for optimal data development.

A. Epidemiologic Studies

The Technical Panel has identified several data gaps that apply to previously conducted epidemiologic studies that are critical to further characterize and estimate the cancer risk for ingested arsenic. These points should be considered in ongoing and future studies:

- level of species of arsenic exposures from all sources (e.g., soil, air, food, cooking water) including drinking water; better characterization of personal habits (e.g., water consumption, pica ingestion) also needed
- further epidemiologic assessment of internal cancers
- rates of Blackfoot disease mortality by age and its effects on the incidence of arsenic-associated cancer
- studies of people who migrate in and out of areas with high levels of inorganic arsenic in drinking water to better ascertain the effects of age and dose on the cancer incidence
- analysis of drinking water supplies for presence of contaminants other than arsenic, with special attention given to ergotamines
- information on diet to determine whether there is a relationship between nutritional status and arsenic-induced cancers
- identification of biological markers (e.g., genotoxicity, liver damage) which correlate with carcinogenic risk

B. Mechanisms of Carcinogenesis for Arsenic-Induced Skin Cancer

Studies are needed to help elucidate the mechanism of arsenic-induced carcinogenicity. Some ideas, which are identified below, have been proposed; however, the Technical Panel acknowledges that these are not all inclusive.

- in vivo studies of clastogenicity and further studies of the mechanisms underlying arsenic-induced genotoxicity
- study of oncogene activation in pre-cancerous and cancerous lesions
- the influence of arsenic on growth factors that may be related to cancer induction

C. Pharmacokinetics/Metabolism of Arsenic

A better understanding of pharmacokinetics and metabolism of arsenic is needed to support the assumptions made with regard to the shape of the

dose-response. It is critical in all such studies that accurate and precise methodology be used and that special attention be paid to sampling because of the potential for interconversion among arsenic species.

- studies on metabolism and patterns of deposition in various tissues for acute and chronic exposure, in humans and animals, for arsenic and its methylated species
- studies on variations in biomethylation in different tissues

D. Essentiality

Elucidation of the role of arsenic in human nutrition will depend on the development of specific information in the following areas:

- biochemical and physiological mechanisms of action
- biological activity and metabolic response to various chemical species of ingested arsenic
- dose-response relationships between animal species

Appendix A

Summary of Epidemiologic Studies and Case Reports on Ingested Arsenic Exposure

Table A-1. Summary of Epidemiologic Studies and Case Reports on Ingested Arsenic Exposure

Author	Type of study	Study population	Results	Highlights/deficiencies
Taiwan				
Astrup, 1968	Case report	Two cases of Blackfoot disease	Both cases lived in an area of Taiwan where there were endemically high levels of arsenic in the water supply.	
Chen et al.,1985	Ecologic correlation	The population of the townships of Peimen, Hsucheia, Putai, and Ichu on the southwest coast of Taiwan. The area is one where the prevalence rate of Blackfoot disease is higher than that of the rest of Taiwan, and where there is an arsenic contamination of artesian wells.	The SMRs for cancers of the bladder, kidney, skin, lung, liver, and colon were 1100, 772, 534, 320, 170, and 160, respectively, for males and 2009, 1119, 652, 413, 229, and 168, respectively, for females. All were statistically significant ($p < 0.05$).	There was a dose-response by type of well used (artesian, shallow, or both) for bladder, kidney, skin, lung, and liver cancer SMRs. NOTE: the artesian wells were contaminated with arsenic; the shallow wells were not. The SMRs for bladder, kidney, skin, lung, and liver cancer correlated with prevalence rates for Blackfoot disease (i.e., the areas with higher Blackfoot disease had higher cancer SMRs).

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Taiwan (continued)				
Chen et al., 1986	Case-control	69 bladder cancer, 76 lung cancer, and 59 liver cancer cases and 368 alive community controls matched as to age and sex were studied to evaluate the association between high-arsenic artesian well water and cancers in the area of Taiwan studied by Tseng (1977) and Chen et al. (1985).	The age-sex-adjusted odds ratios of developing bladder, lung, and liver cancers for those who had used artesian well water for 40 or more years were 3.90, 3.39, and 2.67, respectively, as compared to those who never used artesian well water. Dose-response relationships were observed for all three cancer types by duration of exposure. Multiple binary logistic regression analyses showed that the dose-response relationships and odds ratios remained much the same while other risk factors were further adjusted.	
Ch'i and Blackwell, 1968	Case-control	353 cases of Blackfoot disease and 353 controls matched for sex and age in an area of Taiwan with an endemic arsenic contamination of the water supply.	Significantly ($p < 0.01$) more cases than controls were found to consume deep well water known to be contaminated with arsenic.	Both economic and educational status were significantly lower among the cases than among the controls.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Taiwan (continued)				
Tseng et al., 1968; Tseng, 1977	Cross-sectional	40,421 residents of 37 villages in an area of Taiwan with an endemic arsenic contamination of the drinking water supply.	A skin cancer prevalence rate of 10.6/1,000 for those drinking well water was found, compared to 0/1,000 for a control area. The skin cancer rate followed a dose-response by arsenic concentration in the water.	The physicians who conducted the physical examinations were not "blinded" as to exposed and non-exposed persons. The rate of Blackfoot disease was 360 per 1,000 in the study population vs. 0 per 1,000 in the control population. Blackfoot disease and skin cancer occurred together more often than would be expected if they were random occurrences. Because of the high case fatality rate and lower median age of onset for Blackfoot disease, this may have underestimated the skin cancer risk. The studied population had a protein-deficient diet and poor medical care, both of which might have increased the skin cancer risk.
Central and South America				
Albores et al., 1979	Cross-sectional	Mexico High exposure group: 296 inhabitants of El Salvador de Arriba (mean annual arsenic concentration in water = 0.5 ppm). Low exposure group: 318 inhabitants of San Jose de Vinedo (mean annual arsenic concentration in water = 0.001 ppm).	Rate of palmoplantar hyperkeratosis was 14.8% in high exposure vs. 0.3% in low exposure and for dyschromia--31.7% vs. 3.14%, respectively.	Clinical stages of chronic hydroarsenicism could not be distinguished. 33% of the inhabitants of each town were included. Rates exposure were not age-adjusted, but age distributions of populations at risk were given by authors. No other pathways of exposure nor other causes were suggested.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Alvarado et al., 1964	Cross-sectional	Mexico 476 residents of the colonies of Miguel Aleman and Edwardo Guerra.	61% of population had arsenicism. Highest incidence was in children (5-14 yrs). In 297 cases, 73% were classified as benign arsenicism, 24% with advanced arsenicism, and 3% with chronic arsenicism. Reported arsenic level in colonies' water sources ranged from 0.5 to 3.9 ppm.	No control population. Lack of exposure and disease duration information.
Bergoglio, 1964	Proportionate mortality	Argentina 137,702 deaths in the province of Cordoba between 1949 and 1959.	The proportion of deaths attributed to cancer and malignant tumors (23.8%) was higher in a specific region with high arsenic levels in water compared to cancer deaths (15.3%) in the entire province. Increased proportions of mortality ratios were noted for respiratory and skin cancer in the high-arsenic region. Of all cancer deaths in study locations, 35% were due to respiratory cancer and 2.3% to skin cancer. The proportions in referent population were not provided.	Satellite study performed to validate death certificate data. Lack of arsenic exposure data cited in paper. Proportionate mortality ratios not adjusted for age, sex, or other confounding factors.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Biagini, 1974	Clinical study	Argentina Cases: 14 persons with palmoplantar keratosis and epitheliomas (4 cases had melanoderma) who resided in area with high arsenic concentrations. Controls: 16 persons with no history of residing in area of high arsenic concentrations.	Retention rate of arsenic by thyroid glands was higher in cases. Iodine metabolism same for both groups.	Small sample size. Selection criteria of cases and controls were not described by investigators.
Biagini, 1972	Proportionate mortality	Argentina Study population consisted of 116 patients from Cordoba who were being treated for chronic arsenic poisoning.	Of 78 who died from various causes, 24 died from cancer. The percentage of deaths from cancer was 30.8% compared to the general rate of Cordoba, where the percentage of deaths from cancer is 15%. The rate was significantly high.	No exposure data reported.
Biagini et al., 1978	Case report	Argentina 276 adult patients, primarily from Cordoba and Santiago del Estero.	15 of the 276 (5.4%) patients with symptoms of chronic hydroarsenicism were found to have lung cancer. 11 of 15 were heavy or moderate smokers.	Study was not population-based. No control population was used.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Biagini et al., 1972	Cross-sectional	Argentina 3 groups with 100 male patients over 35 years old in each. Residents of Cordoba.	First group (with arsenic exposure and symptoms of chronic arsenic poisoning) had 23 leucoplasias of the oral cavity, 5 spinocellular cancers, and 2 cancers of the larynx. Second group (those with arsenic exposure and no symptoms of poisoning) had 17 leucoplasias and 1 spinocellular epithelioma. Third group (from area without high arsenic concentrations and no symptoms) had 8 leucoplasias.	Study was not population-based. Confounders or other causes were not studied.
Biagini et al., 1974	Cross-sectional	Argentina 51 persons in Urutau (pop. 210) whose daily activities did not require them to leave village.	Prevalence rate of palmoplantar keratoses was 25.4% (13/51); for epitheliomas, 9.8% (5/51); and for melanoderma, 11.8% (6/51). Water from two local sources had arsenic levels of 0.76 ppm to 0.8 ppm.	Study did not include a control population. Total population at risk may have been underestimated.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Borgono et al., 1980	Cross-sectional	Chile 1,277 children (11-15 yrs old) of northern Chile.	Author reported that the prevalence of cutaneous lesions ranged from 3.5 to 64% in children residing in 5 localities. The rate of lesions was roughly correlated with arsenic levels in drinking water. Levels of arsenic in hair, nail, and urine samples and water supplies exceeded normal values in most cases. No difference was found in hair, nail, and urine levels of arsenic between children with or without skin lesions.	No control group. No specification of disease symptoms or their frequency. Arsenic levels in drinking water supplies of localities presented in article.
Borgono et al., 1977	Cross-sectional	Chile Group A: Antofagasta inhabitants over 6 years of age in 1976 who were exposed to arsenic in drinking water prior to the operation of a water treatment facility. Group B: Antofagasta inhabitants under 6 years of age in 1976 who were not exposed to high arsenic levels.	No cutaneous lesions in the low exposure Group B. Prevalence of lesions in Group A was 15.3% (52/339) in males and females. Abnormal arsenic levels were found in the hair and nail samples of both groups.	Water treatment facility has reduced arsenic levels in drinking water. Arsenic levels were approximately 0.8 ppm prior to 1970 when treatment plant started operations. Disease symptomatology not specified. Causative factors other than the construction of a filter plant in 1970 were not considered.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Borgono and Greiber, 1972	Cross-sectional	Chile High-exposure group: 204 residents of Antofagasta. Control group: 96 residents of Iquique.	Antofagasta residents had abnormal skin pigmentation and a mean arsenic level of 0.61 mg/100 g in hair, and subjects with normal skin had an arsenic level of 0.32 mg/100 g in hair. Iquique residents had no abnormal pigmentation and the mean arsenic level in hair was 0.08. The prevalence rate, among Antofagasta residents, of abnormal skin pigmentation and hyperkeratosis was 80% and 36%, respectively. Neither condition occurred in Iquique residents.	No exposure data. Sex distribution differs for exposure groups. Selection criteria were not explained by authors.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Cebrian et al., 1983	Cross-sectional	Mexico Exposed population: 296 of 998 (29.6%) inhabitants of El Salvador de Arriba where arsenic levels in drinking water were 0.41 ppm. Control population: 318 of 1,488 (21.4%) persons from San Jose del Vinedo with arsenic concentration in drinking water of 0.005 ppm.	Prevalence rate of cutaneous signs of arsenic poisoning was 21.6% (64/296) in exposed population vs. 2.2% (7/318) in control population. Prevalence rates of specific conditions in exposed population were 17.6% (52/296) hypopigmentation, 12.2% (36/296) hyperpigmentation, 11.2% (33/296) palmoplantar keratosis, 5.1% (15/296) papular keratosis, and 1.4% (4/296) ulcerative zones. All of these rates were significantly greater than those in control population at $p < 0.05$. Relative risks of palmoplantar keratosis and hyperpigmentation were 36.0 and 6.4, respectively. Minimum total dose for skin lesions was 2 g for hypopigmentation, 3 g for hyperpigmentation and palmoplantar keratosis, 8 g for papular keratosis , and 12 g for ulcerative lesions. Shortest latency period for hypopigmentation was 8 years; for hyperpigmentation or palmoplantar keratosis, 12 years; for papular keratosis, 25 years; and for ulcerative lesions, 38 years.	Study subjects selected by a systematic sampling scheme of households. Study populations derived from communities with similar socioeconomic conditions and age and sex distributions. Minimum total doses calculated for specific dermal lesions were not adjusted for body weight or daily consumption of arsenic. Papular keratosis and ulcerative lesions were probably carcinomas. Latency periods for dermal lesions may have been subject to recall bias or study artifacts due to use of prevalence data. 70% of arsenic in drinking water of exposed population was in pentavalent form; the remainder was in trivalent form.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Chavez et al., 1964	Cross-sectional	Mexico 291 residents (57.6%) of community of Finisterre.	38.8% (114/291) of the studied population demonstrated symptoms of chronic arsenic poisoning. Prevalence of spotty hyperkeratosis was 66% (92/291); hyperpigmentation, 12.4% (36/291); and carcinoma, 0.3% (1/291). Poisoning symptoms were not present in subjects younger than 7 years. In subjects over 10 years of age, symptoms occurred more frequently in males than in females. Frequency of disease increased with age, years of residency, and nutritional deficiency. Prevalence rate of various indices of nutritional status in cases with chronic poisoning was greater than those without disease.	Detailed classification of symptomatology and investigation of socioeconomic and nutritional factors in the sample. No control group and no exposure data. Insufficient data to determine if poor nutritional status preceded onset of disease.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Sanchez de la Fuente, undated	Cross-sectional	Mexico 6,287 (3,179 males, 3,108 females) from 17 rural communities from 1962 through 1964.	Study covered 6,287 of 7,271 (86%) persons at risk. 5.3% (335 cases) of the sample exhibited clinical signs of chronic arsenicism. Symptoms had been present for 1-4 years in over half of the cases. Prevalence of symptoms included: 5.0% (317/6,287) hyperkeratosis, 4.0% (252/6,287) melanoderma and dischromia, 2.9% (183/6,287) hyperdrosis, 2.4% (152/6,287) nail deformation, and 0.05% (3/6,287) epidermoid carcinoma. Some wells in study area had arsenic levels of 0.09 to 0.65 mg/L.	No specific exposure data. Disease rates were apparently not age-adjusted.
Zaldivar, 1974	Cross-sectional	Chile Survey of 457 patients (208 males, 249 females) from Antofagasta with hydroarsenicism lesions reported between 1968 and 1971. Comparison of arsenicism rates in Antofgagasta before and after introduction of a water treatment facility in 1970.	Arsenicism was most prevalent in children. Arsenic dose decreased linearly as patient age increased. Yearly mean arsenic concentrations in drinking water were positively correlated with incidence rates between 1968 and 1971. Lesions included leukoderma, melanoderma, hyperkeratosis, and squamous cell carinoma. The mean arsenic concentration of drinking water.	Symptoms were not specified according to dose.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Zaldivar, 1974 (continued)			samples from 1968 was 0.58 ppm, and arsenicism incidence (per 100,000 population) was 146 for males and 168 for females. In 1971, the mean arsenic level was 0.08 ppm, and incidence rates declined to 9 and 10 (per 100,000) for males and females, respectively.	
Zaldivar and Guillier, 1977	Case report	Chile 470 patients (220 males, 250 females) from Antofagasta with arsenicism-associated dermatosis between 1968 and 1971.	Of the 470 patients, 50.2% were under the age of 10 years and 76.6% were under the age of 20 years. High levels of arsenic were found in the hair and nails, but not in urine of patients exposed in 1968. Five children out of 337 cases (0-15 yrs) died and autopsies indicated fibrous intimal thickening of arteries, epidermal atrophy, dermal fibrosis, and hyperkeratosis. Estimated yearly mean doses of ingested arsenic for the deceased children ranged from 0.128 mg/kg bw/day for the first year to 0.028 mg/kg bw/day in the seventh year.	Selection bias may have existed since cases originated from a hospital. Arsenic dosage in sick children was extrapolated from ingestion experience of healthy children during 1972.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Central and South America (continued)				
Tovar et al., 1964	Clinical	Mexico 12 of 294 persons from the community of Finisterre with varying degrees of arsenicism, including 3 persons without the disease, received calcium trisodium diethyl-tetra-amino-penta acetate (DTPA) to test the efficacy of the drug in eliminating body burden of arsenic.	Administration of DTPA did not result in excretion of arsenic.	Investigators reported that cases came from community with arsenic concentration in drinking water ranging from 0.6 to 0.9 ppm. Small sample size. All persons in study were from same area and similar backgrounds.
Zaldivar, 1977	Cross-sectional	Chile Dietary and water intake survey of 220 persons in 1972 representing nine age groups of each sex. Arsenicism prevalence rates were developed from another population (i.e., Antofagasta Commune) for 1968 through 1971.	Arsenic dose levels were inversely related to age and ranged from 0.0022 to 0.0633 mg/kg/day. Age-specific prevalence rates of chronic arsenic poisoning ranged from 0 to 726 per 100,000 and were positively correlated with age-specific arsenic doses. Children (0-15 yrs) had more severe symptoms and higher ingested arsenic doses. Lesions included leukomelanoderma, hyperkeratosis, and multiple squamous cell carcinoma.	Very few studies have reported dose-response data. Exposure was based on 1972 data and disease rates from 1968 through 1971. Arsenicism symptoms were not specified according to dose.

(continued)

***Table A-1.* (Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Germany				
Geyer, 1898	Case reports	Individuals in mining region in Silesia drinking ground water containing arsenic.	Kerotoses and melanoses reported in approximately 20 individuals in one village who consumed contaminated water. Several cases were reported in families. Individuals in broader region were reported to have short lifespan. Nervous disturbances similar to those observed by Hutchinson were noted. Reports of three individuals who developed skin cancer, attributed to arsenic, are presented. In recent years, water supply had been replaced and health problems lessened.	Information limited to case reports; however, these reports represent careful clinical observations of related problems in individuals in a small geographic area.
Liebegott, 1952	Autopsy series	Wine growers exposed to arsenic from pesticides. Author concludes that principal arsenic exposure was through homemade drink from grapeskins reported to contain arsenic at up to 5 mg/L.	In a series of 19 autopsied growers, all were found to have arsenical hyperkeratosis on hands and soles of feet. 17 had liver cirrhosis attributed to arsenic. Three of the latter had multicentric liver carcinomas. Two additional cases with liver carcinoma were examined. An additional group of 8 cases of skin carcinoma in conjunction with hyperkeratosis were reported. In 5 patients, multiple carcinomas were seen; in untreated cases, regional lymph node metastases were seen.	The size of the population from which autopsied cases were drawn was unspecified, as were reasons leading to autopsy.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Germany (continued)				
Luchtraht, 1972	Autopsy series	Moselle vintners exposed to arsenic pesticides. Exposures ceased in 1942; autopsies were performed from 1960 to 1977. Autopsied cases were stated to have had chronic arsenic poisoning.	Among 163 patients, 108 with lung tumors (66%), 30 (18%) with skin carcinomas, 54 (33%) with Bowen's disease, and 5 (3%) with liver tumors were noted. For comparison, 163 age- and sex-matched postmortem examinations in non-wine growers were reviewed. In that group, 14 lung cancers (14%) and no tumors of the other types listed above were noted. Additional data from a local trade association registry of 417 wine grower deaths contained similarly high excesses of lung and liver cancer (skin not mentioned). Skin hyperkeratoses were also a prominent finding in author's examinations being found in almost all those examined.	Analysis of data is limited by lack of clearly defined method of selection of autopsied cases. Reported skin, lung, and liver tumor occurrences were strikingly high.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Germany (continued)				
Roth, 1957	Autopsy series	Moselle vintners exposed to arsenic trioxide were autopsied to ascertain whether arsenic exposure led to death. Vintners had been exposed to insecticides for 12 to 17 years, with death occurring 8 to 14 years after cessation of exposure.	Pathologic findings were reported for 27 autopsies. 16 patients had a total of 28 malignant neoplasms, including 12 cases with bronchial carcinomas, 5 cases with skin carcinomas, and 3 with liver tumors. Hyperkeratoses were prominent in the group, 13 cases of liver cirrhosis attributed to arsenic were noted, and 1 individual had peripheral vascular damage leading to amputation of a leg.	The size of the population from which the autopsies were drawn is not specified; neither are the specific circumstances that led to an autopsy being performed.
Roth, 1956	Autopsy series	See Roth (1957).	Provides greater detail on 24 of the autopsies reported in Roth (1957). Estimates of arsenic exposure levels are presented.	See Roth (1957).

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
United States				
Birmingham et al., 1965	Community medical survey	Residents near gold smelter which produced substantial arsenic dust. Limited water samples showed 0.03 mg/L arsenic.	32/40 school children showed "suspect arsenical dermatoses." Ulceration was noted on hands. Several housewives also were afflicted with skin problems.	
Harrington et al., 1978	Survey of symptom prevalence and levels of arsenic exposure	232 residents in Fairbanks, Alaska, divided into 4 groups according to drinking water source, e.g., bottled water or high-arsenic ground water.	A correlation between water arsenic level and urine arsenic level was demonstrated. The authors stated that information obtained by questionnaire and clinical exams did not demonstrate any intergroup differences in skin, peripheral nervous system, or other abnormalities.	The sizes of examined groups were small; exposure was less than 10 years in duration; no data on the clinical observations of symptoms was reported.
Kjeldsberg and Ward, 1972	Case report	Women who used arsenical pesticides in gardening.	Patient developed pancytopenia and later, melogenous leukemia. Physicians believed illness was arsenic-related.	Single case report.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
United States (continued)				
Southwick et al., 1983	Clinical examination, disease incidence and mortality analysis, and exposure assessment	Populations of Hinckley and Desert, Utah, who were exposed to approximately 0.2 mg/L arsenic in drinking water. Population of nearby Delta (< 0.25 mg/L arsenic) served as control.	Elevated urine arsenic demonstrated. Statistically elevated prevalence of dermatological signs or other symptoms was not observed. Hinckley showed relatively high total cancer mortality data, but cancer incidence data was not similarly high (neither of which had any bearing on skin cancer).	Very small population studied (144 total for Hinckley and Desert; 31 age 60 or older given physical exam). Andelmann and Barnett (1984) calculated that the negative findings were not inconsistent with the EPA risk model based on Taiwan data.
Wagner et al., 1979	Case report	41-year-old woman who was consuming well water containing 1.2 ppm arsenic in Lane County, Oregon.	12 years previously, patient had been diagnosed as having acute arsenism after drinking contaminated well water for 6 months. Authors reported that she had multiple skin lesions (43 were removed), including *in situ* squamous cell carcinoma and multicentric basal cell carcinoma.	
Morton et al., 1976	Geographic correlation of skin cancer incidence with measured drinking water arsenic levels	Lane County, Oregon, population: 190,871 in 1965. Skin cancer incidence determined from pathology records; arsenic levels measured in 558 water samples, 8% of which exceeded 50 ppb.	No relation was found in correlation between district skin cancer and average arsenic levels.	Age standardization was accomplished by an indirect regression method. Andelmann and Barnett (1984) calculated the negative findings by Morton et al. and concluded that they were not inconsistent with the EPA risk model based on Taiwan data.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
United States (continued)				
Kelynack et al., 1960	Clinical observation and analysis of mortality records	Beer drinkers exposed to arsenic through contaminated ingredient. Chemical measurements of arsenic in beer were made (trace - 4.8 ppm, average 1.7 ppm in 16 samples).	Physicians noted unusual excess of patients with peripheral neuritis manifested in weakness and pains in limbs and difficulty in walking. Patients also typically had skin disorders: darkening, thickening, and deterioration of skin on hands and feet, many cases of "branny" desquamatization. Review of mortality records revealed that deaths attributed to neuritis or alcoholism totaled 66 in 4-month period of poisoning episode, compared with 27 to 39 in previous whole years.	
England				
Philipp et al., 1983	Geographic correlation	Cancer registry data on cases of malignant melanoma were obtained by district in southwest England. Population data were available by census. A nationwide survey of arsenic levels in stream-bed sediments was used to classify districts into high- and low-arsenic categories.	A positive correlation ($p < 0.05$) was found between district melanoma rates and arsenic categories in males. When analysis was restricted to rural areas, the positive correlation was again obtained. No similar correlation was found in females, who had higher overall incidence rates than males.	No common trend was seen in males and females. The arsenic measure used may or may not be reflective of population arsenic exposure.

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
England (continued)				
Reynolds et al., 1901	Review of clinical experience	Residents of Manchester who consumed beer contaminated with arsenic. Chemical measurements revealed 2 to 4 ppm arsenic in beer.	Author had charge of 500 patients with arsenical poisoning, of whom 13 died. Skin lesions were present in almost all patients. Skin darkening, keratosis of hands and feet, herpes zoster, and presence of tender, irritated regions were common. Patients experienced loss of feeling and weakness in limbs. Circulatory problems were noted. Author estimates at least 2,000 cases of poisoning occurred in Manchester.	
China				
Yue-zhen et al., 1985	Clinical survey of community	359 persons residing at an industrial plant consumed water containing 0.6 mg/L arsenic.	Among 336 individuals examined, 150 with skin lesions of chronic arsenism were found. All affected had consumed the contaminated water for 6 months to 12 years. 126 cases of dyspigmentation and 84 keratotic lesions (primarily on palms and soles) were noted. Among 33 patients questioned in depth, 13 noted numbness, most commonly on hands and feet; a variety of other symptoms were noted. The authors reported that 2 cases of cutaneous carcinoma had been reported in the same area; however, none were observed in this study.	

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Japan				
Yamashita et al., 1972	Study of teenagers who were exposed to arsenic-tainted milk in infancy. Parental interviews, physical exams, and psychological tests were administered to assess state of health and development.	554 exposed teenagers were identified from a variety of local sources. Controls came from the exposed students' schools. Controls for psychological tests came from one local school. No controls were used in physicals.	Parents reported that exposed children had a variety of physical maladies and learning/social difficulties. Many complaints, including dark spots and white spots on skin, were well in excess of controls. Medical exam identified central nervous system problems, skin problems (hyperpigmentation, depigmentation, hyperkeratosis [15%]), low height, and other symptoms (no statistics). Intelligence tests and other psychological tests showed markedly poor performance for exposed group compared with the local high school.	Study approaches were not as refined as are needed for good statistical comparisons. Study identified numerous problems which deserve more attention. Large Japanese exposed group is available for future work.
Arsenical Medicinals				
Braun, 1958	Case report	18 patients with skin and/or visceral cancers.	16 of the patients with skin and/or visceral cancer were vintners who had used arsenical pesticides; one patient with skin and lung cancer had taken an arsenical medicinal; another skin cancer patient had taken an arsenical medicinal.	

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Arsenical Medicinals (continued)				
Cuzick et al., 1982	Cohort	478 patients treated with Fowler's solution (an arsenical medicinal) for periods ranging from 2 weeks to 12 years between 1945 and 1969.	A statistically significant association of ingestion of Fowler's solution with deaths from internal malignancies was not found. A subset of 142 of the 478 in the cohort was examined in 1969-1970, and 49% were found to show dermal signs of arsenicism, including skin cancer (11%).	In the subset of 142, deaths from internal malignancies occurred only in those who had previously demonstrated dermal signs of arsenicism, leading the authors to conclude that perhaps persons who show signs of arsenicism are at a greater risk of death from internal malignancies. Patients with signs of arsenicism (keratosis, hyperpigmentation, and skin cancer) had higher median doses than those without signs.
Falk et al., 1981	Case report	7 male patients and 1 female angiosarcoma patient.	All of the patients had previously taken Fowler's solution.	
Fierz, 1965	Cohort	262 patients treated with Fowler's solution by a private practitioner.	106 of the 262 patients reporting for physical examination reported hyperkeratosis; 21 cases of skin cancer were found. The response increased with increasing dose.	Less than 45% of a group of 1450 to whom invitations to participate in the study were sent presented themselves for physical examination, and the author himself reported that the patients reporting for examination were not a representative sample. No controls were used.

(continued)

Table A-1. **(Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Arsenical Medicinals (continued)				
Hutchinson, 1888	Case report	6 patients being treated with arsenical medicinals	The 6 patients treated with arsenical medicinals exhibited the keratotic lesions associated with arsenical poisoning.	
Istvan et al., 1984	Case report	Individual who received 7-month course of arsenic therapy for psoriasis	Angiosarcoma of liver developed 25 years after therapy.	
Jackson and Gainge, 1975	Case report	7 individuals tested with Fowler's solution. Cases selected from clinical files.	Six cases of basal cell carcinoma, carcinoma *in situ*, or squamous cell carcinoma of the skin were reported. Two of these patients had systemic cancer (breast and colon). All 7 patients showed keratosis on palms and soles.	
Knoth, 1966	Case report	2 male patients, one with a reticulosarcoma of the glans penis and one with skin cancer; one female patient with both skin cancer and mammary cancer.	All of these patients had previously been treated with Fowler's solution.	
Lander et al., 1975	Case report	1 male angiosarcoma patient	The patient had previously taken Fowler's solution.	

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Arsenical Medicinals (continued)				
Morris et al., 1974	Case report	2 male patients; one had skin pigmentation, skin tumors, carcinoma of the larynx, and a probable bronchial carcinoma; the other had skin pigmentation and keratosis. Both had non-cirrhotic portal hypertension.	Both patients had previously taken Fowler's solution.	
Neubauer, 1947	Case report	143 patients with epithelioma.	The patients had previously been treated with arsenical medicinals for skin diseases and various internal disorders.	
Nurse, 1978	Case report	Male patient with adenocarcinoma of the kidney.	Patient had take arsenical medicinal approximately 20 years previously for psoriasis.	Patient treated with a variety of other drugs before developing kidney cancer.
Popper et al., 1978	Case report	4 male and 1 female angiosarcoma patients.	Four of the patients had previously taken Fowler's solution for 10 to 17 years for psoriasis or asthma. There was not enough information for the fifth patient to ascertain duration of exposure.	

(continued)

Table A-1. (Continued)

Author	Type of study	Study population	Results	Highlights/deficiencies
Arsenical Medicinals (continued)				
Prystowsky et al., 1978	Case report	Female patient with nasopharyngeal cancer and with palmar and plantar keratosis.	Patient has received Fowler's solution almost yearly for 20 years prior to development of the cancer.	
Regelson et al., 1968	Case report	Male patient with hemangioendothelial sarcoma of the liver.	The patient had previously been treated with Fowler's solution for psoriasis for 17 years.	
Reymann et al., 1978	Cohort	389 patients treated with arsenical medicinals between 1930 and 1939 at a dermatology clinic in Denmark.	Of 389 persons treated with arsenical medicinals, 41 internal malignant neoplasms were found to occur during 1943 through 1974 versus 44.6 expected. No increase in internal malignant neoplasms was found by dose.	The end point, "internal malignant neoplasms," is not specific. It is possible that the risk of cancer for particular organ sites was elevated, but this was not reported by the authors.
Roat et al., 1982	Case report	Male patient with angiosarcoma and skin cancer.	Patient had previously ingested Fowler's solution for 6 months.	

(continued)

***Table A-1.* (Continued)**

Author	Type of study	Study population	Results	Highlights/deficiencies
Arsenical Medicinals (continued)				
Sommers and McManus, 1953	Case report	27 cases of skin cancer; 10 of the cases also had visceral cancer.	12 of the patients had been treated with Fowler's solution for psoriasis, 2 for epilepsy, and 1 with injected arsenicals for syphilis. 2 patients had occupational exposure to arsenical sprays and 4 had possible exposures. 7 had been treated with arsenic for swollen lymph nodes, burns, dermatitis, or chorea. In 3 patients the means of exposure were unknown. All had the characteristic keratosis of the palms and soles.	For the 22 patients for whom the time from beginning of exposure to onset of cancer was known, the latent period ranged from 3 to 50 years.

Appendix B

Quantitative Estimate of Risk for Skin Cancer Resulting from Arsenic Ingestion

Contents

Tables

Figures

I. Methodology

A generalized multistage model is employed to predict the prevalence of skin cancer as a function of arsenic concentration in drinking water (d) and age (t), assuming exposure to a constant dose rate since birth. Let F(t,d) represent the probability of developing skin cancer by age t after lifetime exposure to arsenic concentration d. The model has the following form:

$$F(t,d) = 1 - \exp[-g(d)\,H(t)]$$

where g(d) is a polynomial in dose with non-negative coefficients, and H(t) is $(t-w)^k$, where k is any positive real number, and $t > w$ for induction time w. The model F(t,d) is a generalization of the multistage in which k can only assume the value of positive integers. The multistage model is consistent with the somatic mutation hypothesis of carcinogenesis (Armitage and Doll, 1954; Whittemore, 1977; Whittemore and Keller, 1978). It also results from the epigenetic hypothesis when reversible cellular changes occur randomly (Watson, 1977). Moreover, it can be derived from the multistage theory of carcinogenesis (Armitage, 1982). These authors and many others have used this model to interpret and/or estimate potency from human data. The number of people at risk and the number with skin cancer at different values of t and d must be known in order to employ maximum likelihood estimation (MLE).

II. Application to Taiwan Epidemiologic Study

In order to use the model described above and the prevalence data provided by Tseng et al. (1968) and Tseng (1977), the following three assumptions must be made:

(1) The mortality rate was the same in the diseased (skin cancer) persons as in the nondiseased persons.

(2) The population composition (with respect to the risk factors of the skin cancer) remained constant over time. This assumption implies that there was no cohort effect.

(3) The skin cancer was not surgically removed.

The first assumption may not be reasonable because there is reason to believe that the mortality rate in the diseased (skin cancer) persons was higher than in the nondiseased persons. Tseng et al. (1968) reported that 61 skin cancer patients (out of a total of 428 individuals with skin cancer) had also incurred Blackfoot disease which was known to have higher death rates than the general population. The impact of this potential differential mortality will be investigated in Section V of this Appendix. The second assumption seems less a problem in view of the fact that the population studied by Tseng and his associates was stable. However, the probability still exists that there may be some cohort effect due to the change of risk factors, such as the change of the arsenic water concentration over time (over 60 years). The last assumption is reasonable because the studied population was very poor, and medical (surgical) service to the population was almost nonexistent.

Tseng et al. (1968) and Tseng (1977) reported skin cancer prevalence rates as percentages specific to age group and arsenic concentration for each gender. The underlying "raw" prevalence ratios were calculated from the percentage estimates by use of data in Tseng's 1968 publication. The use of these ratios permits use of all the data, including that for controls and the 0 to 19 age group, which had not been included in EPA's 1984 analysis. The

procedure used for estimating the actual number of persons at risk is presented in the paragraphs that follow.

The percentage age distribution of the population in the endemic area by gender appears in Table 3 of Tseng et al. (1968). (Note that the percentages for males and females in the endemic area do not sum to 100.) Age group percentages were applied to the male population surveyed (19,269) to estimate the totals at each age. These were distributed among the four dose categories under the assumption that the age distribution of the surveyed males at each arsenic exposure category is the same. This was accomplished by solving a set of equations. Table B-1 shows the resulting distribution of the male population at risk. Furthermore, it was assumed that the distribution of surveyed females across age and dose categories was the same as that for men (see Table B-2). The age distribution of the control population appears in Table 3 of Tseng et al. (1968). Tables B-1 and B-2 also show the number of cancer cases observed in each age and dose group.

Next, values of t and d representative of each age and arsenic concentration interval were determined. For each interval a weighted average age was calculated from the data in Table 3 of Tseng et al. (1968). The resulting values of t that relate to the skin cancer prevalence rate for males (females) are 8 (9), 30 (30), 49 (50), and 69 (68).

From the distribution of arsenic concentrations in well water depicted in Figure 2 of Tseng et al. (1968), and the fact that the highest arsenic content in surveyed well water was 1.82 ppm, weighted average arsenic concentrations (in ppm) of 0.17, 0.47, and 0.80 were calculated for the low, medium, and high concentration groups, respectively. (This approach does not accommodate the variation with respect to time of the arsenic concentration in well water noted by the authors, but for which no data are available.) These values were then converted into equivalent doses for the U.S. person in units of μg/kg/day using the following assumptions: the "reference" U.S. person weighs 70 kg and consumes 2 L of water daily; the "reference" Taiwanese male weighs 55 kg and consumes 3.5 L of water daily; and the "reference" Taiwanese female weighs 50 kg. The resultant arsenic dose rates, normalized to the reference U.S. person, are presented in Table B-3.

These data were used with the generalized multistage model to predict dose- and age-specific skin cancer prevalence rates associated with ingestion of inorganic arsenic for the reference U.S. person based on the Taiwanese experience. The four dose groups include control, low, medium, and high.

The model was fitted separately to the skin cancer data for males and females. The g(d) was evaluated as to linear and quadratic function of dose (i.e., two models were considered; one was linear in dose and the other was both linear and quadratic in dose). The MLEs of g(d), H(t), and the log likelihood (ln L) estimate are shown in Table B-4. Table B-4 shows the unit risk, the probability that a U.S. person exposed to dose d = 1 μg/kg/day of arsenic in drinking water will develop skin cancer in lifetime. It is adjusted for the survivorship of the U.S. population by the life-table analysis.

For visual inspection of the goodness-of-fit of the model with time, values of the observed skin cancer prevalence rates for Taiwanese males were given in Figures B-1 and B-2, for linear and quadratic dose, respectively. Figures B-3 and B-4 show the analogous plots for females. While the suitability for a particular model is not obvious from these plots, there is some evidence favoring the quadratic (both linear and quadratic in dose) model. For each gender-specific set of models, a test of the null

Table B-1. **Estimated Distribution of the Surveyed Male Population at Risk (Skin Cancer Cases) by Age Group and Concentration of Arsenic in Well Water in Taiwan[a]**

Arsenic concentration (ppm)	Age group (years)				
	0-19	20-39	40-59	≥60	Total
Low (0-0.30)	2,714[b] (0)[c]	935 (1)	653 (4)	236 (11)	4,538 (16)
Medium (0.30-0.60)	1,542 (0)	531 (2)	371 (18)	134 (22)	2,578 (42)
High (> 0.60)	2,351 (0)	810 (18)	566 (56)	204 (52)	3,931 (126)
Unknown	4,933 (0)	1,699 (3)	1,188 (61)	429 (64)	8,249 (128)
Total	11,540 (0)	3,975 (24)	2,778 (139)	1,003 (149)	19,296 (312)

[a]For the control group, the number of persons in each of the four age groups, 0-19, 20-39, 40-59, and ≥ 60, are respectively 2,679, 847, 606, and 176. No skin cancer was observed in the control population.
[b]Estimated number of persons at risk.
[c]Estimated number of skin cancer cases observed.

Table B-2. **Estimated Distribution of the Surveyed Female Population at Risk (Skin Cancer Cases) by Age Group and Concentration of Arsenic in Well Water in Taiwan[a]**

Arsenic concentration (ppm)	Age group (years)				
	0-19	20-39	40-59	≥60	Total
Low (0-0.30)	2,651[b] (0)[c]	1,306 (0)	792 (3)	239 (2)	4,988 (5)
Medium (0.30-0.60)	1,507 (0)	742 (1)	450 (9)	136 (8)	2,835 (18)
High (> 0.60)	2,296 (0)	1,131 (4)	686 (33)	207 (22)	4,320 (59)
Unknown	4,819 (0)	2,373 (2)	1,440 (13)	435 (27)	9,067 (42)
Total	11,273 (0)	5,552 (7)	3,368 (58)	1,017 (59)	21,210 (124)

[a]For the control group, the number of persons in each of the four age groups, 0-19, 20-39, 40-59, and ≥ 60, are respectively 2,036, 708, 347, and 101. No skin cancer was observed in the control group.
[b]Estimated number of persons at risk.
[c]Estimated number of skin cancer cases observed.

Table B-3. **Conversion of Arsenic Dose for Taiwanese to Equivalent Arsenic Dose for U.S. Populations[a]**

	Taiwanese (ppm)	U.S. person (μg/kg/day)
Males	0.17	10.8
	0.47	29.9
	0.80	50.9
Females	0.17	6.8
	0.47	18.8
	0.80	32.0

[a]Assumptions: A U.S. person weighs 70 kg and drinks 2 L of water daily; a Taiwanese male weighs 55 kg and drinks 3.5 L of water daily; a Taiwanese female weighs 50 kg and drinks 2 L of water daily.

hypothesis that the coefficient corresponding to d^2 is zero is rejected at $p < 0.01$ via the asymptotic likelihood ratio test.

The estimated induction period (w), based on the experience of Taiwanese males, is approximately 6.9 years, and the estimated power of t is 2.9 (see Table B-4). Analogous estimates from Taiwanese females are 9.0 years and 3.2. The risk for skin cancer estimated from the quadratic model (2×10^{-3} and 1×10^{-3} per μg/kg/day) for males and females, respectively, is smaller than that estimated from the linear model (5×10^{-3} and 3×10^{-3} per μg/kg/day). With each model, the estimated risk for females is slightly less than the corresponding risk for males. Two reasons may explain why the risk estimate calculated on the basis of data for Taiwanese males is greater than that calculated on the basis of data for Taiwanese females: (1) the daily water consumption by Taiwanese males (3.5 L/day) in relation to that consumed by females (2 L/day) may be underestimated; and (2) males, in particular those who were healthy, were more likely than females to migrate out of town, and thus were not available at the time of the survey.

The current U.S. drinking water standard for arsenic is 50 μg/L, which is equivalent to 1.4 μg/kg/day for the reference U.S. person. Figures B-5 and B-6 are plots of lifetime risk of skin cancer for a U.S. reference person as predicted from the model using the gender-specific Taiwan data. At 50 μg/L, the lifetime risk is estimated to range from 1×10^{-3} (based on data from Taiwanese females) to 3×10^{-3} (based on data from Taiwanese males) for a 70-kg person who drinks 2 L/day of water contaminated with 50 g/L of arsenic.

Lastly, age- and gender-specific nonmelanoma skin cancer incidences among Singapore Chinese (IARC, 1976) were used in the risk assessment as estimates of background skin cancer rates for Taiwan. The background rates for the four age groups, 0 to 19, 20 to 39, 40 to 59, and ≥ 60 are, respectively, 0, 8.0×10^{-5}, 6.7×10^{-4}, and 3.6×10^{-3} for males, and 0, 7.0×10^{-5}, 5.5×10^{-4}, and 1.1×10^{-3} for females. The purpose of using Singapore rates was to address the comment made by Margolis December

Figure B-1. Observed and predicted skin cancer prevalence for Taiwanese males at three exposure levels, by age; prevalence predicted by use of the model, linear in dose.

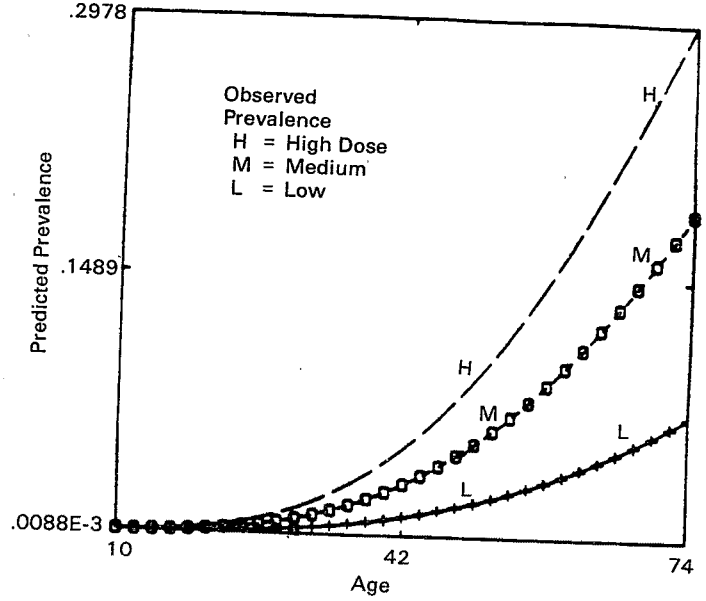

Figure B-2. Observed and predicted skin cancer prevalence for Taiwanese males at three exposure levels, by age; prevalence predicted by use of the model, linear and quadratic in dose.

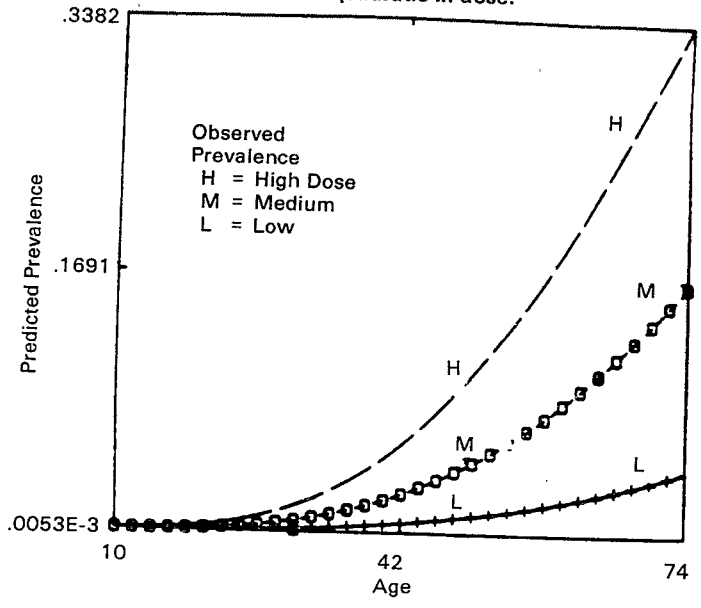

Figure B-3. Observed and predicted skin cancer prevalence for Taiwanese females at three exposure levels, by age; prevalence predicted by use of the model, linear in dose.

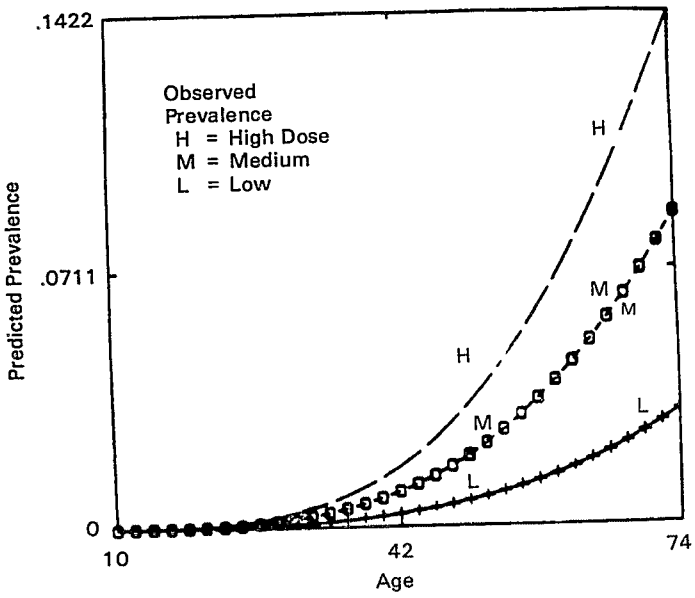

Figure B-4. Observed and predicted skin cancer prevalence for Taiwanese females at three exposure levels, by age; prevalence predicted by use of the model, linear and quadratic in dose.

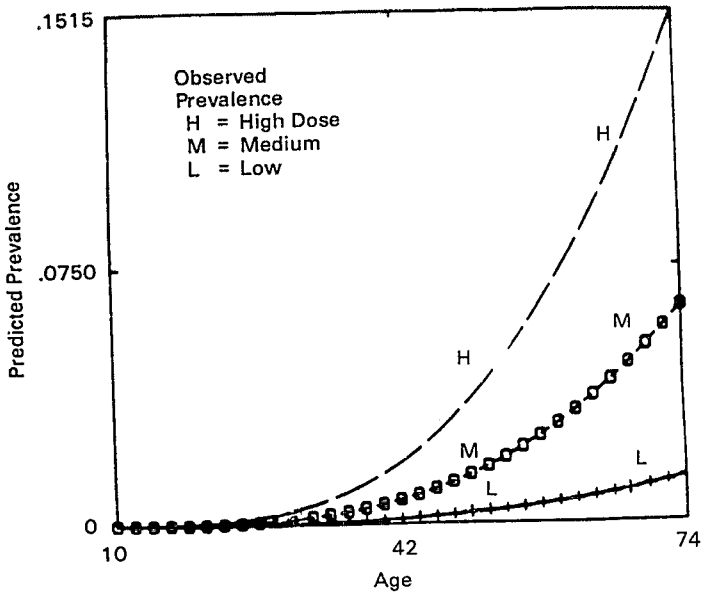

Table B-4. **Results of Model Fitting to Taiwan Skin Cancer Data**

	Linear	Quadratic
Males:		
	Doses (d): 0, 10.818, 29.909, 50.909 µg/kg/day[a]	
	$g(d) = (0.302525 \times 10^{-7})d$	$g(d) = (0.124707 \times 10^{-7})d + 0.404871 \times 10^{-9})d^2$
	$H(t) = (t - 6.931)^{2.935}$	$H(t) = (t - 6.873)^{2.950}$
	$\ln L = -614.551$	$\ln L = -610.088$
	Unit risk (probability of skin cancer in lifetime due to 1 µg/kg/day of arsenic) $= 5.0 \times 10^{-3}$	Unit risk (probability of skin cancer in lifetime due to 1 µg/kg/day of arsenic) $= 2.3 \times 10^{-3}$
Females:		
	Doses (d): 0, 6.8, 18.8, 32.0 µg/kg/day[a]	
	$g(d) = (0.682262 \times 10^{-8})d$	$g(d) = (0.157281 \times 10^{-8})d + 0.204076 \times 10^{-9})d^2$
	$H(t) = (t - 9.0)^{3.225}$	$H(t) = (t - 9.0)^{3.231}$
	$\ln L = -348.041$	$\ln L = -344.365$
	Unit risk (probability of skin cancer in lifetime due to 1 µg/kg/day of arsenic) $= 3.4 \times 10^{-3}$	Unit risk (probability of skin cancer in lifetime due to 1 µg/kg/day of arsenic) $= 1.0 \times 10^{-3}$

[a]Dose estimates for U.S. persons (see Table B-3).
SOURCE: Data from Tseng et al., 1968.

17, 1985 (Letter from Dr. Stephen Margolis, Ph.D., Centers for Disease Control, to Mr. Robert Dupuy, Director, Waste Management Division, U.S. EPA Region 8) that the lack of skin cancer found in the comparison population of 7,500 was anomalous. All Chinese populations for which skin cancer is reported have some incidence of skin cancer. The results of model fitting to the Taiwan skin cancer data, adjusted for this background rate, appear in Table B-5. Comparison of the unit risk estimates in Tables B-4 and B-5 shows that this adjustment is inconsequential. Therefore, the final risk estimate used the background rate reported by Tseng et al. (1968).

III. Use of the Mexican Data to Evaluate Taiwan's Dose-Response Model

Cebrian et al. (1983) studied persons residing in two rural Mexican towns, one with arsenic-contaminated drinking water. The prevalence of skin tumors observed by Cebrian was compared with rates predicted by use of the parameters estimated from Taiwanese data (see Section II of this Appendix). These calculations are discussed below.

Cebrian et al. (1983) published age-specific prevalence rates of ulcerative lesions and papular keratosis among the surveyed groups (see Table B-6).

Figure B-5. Lifetime skin cancer risk for a U.S. person, predicted from the Taiwanese male experience. ''Linear'' = estimated by use of the model, linear in dose; ''Quadratic'' = estimated by use of the model, linear and quadratic in dose.

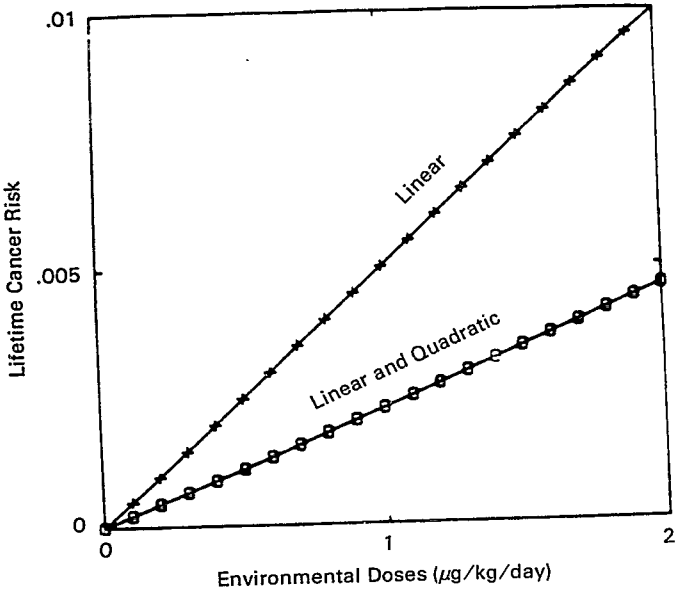

Figure B-6. Lifetime skin cancer risk for a U.S. person, predicted from the Taiwanese female experience. ''Linear'' = estimated by use of the model, linear in dose; ''Quadratic'' = estimated by use of the model, linear and quadratic in dose.

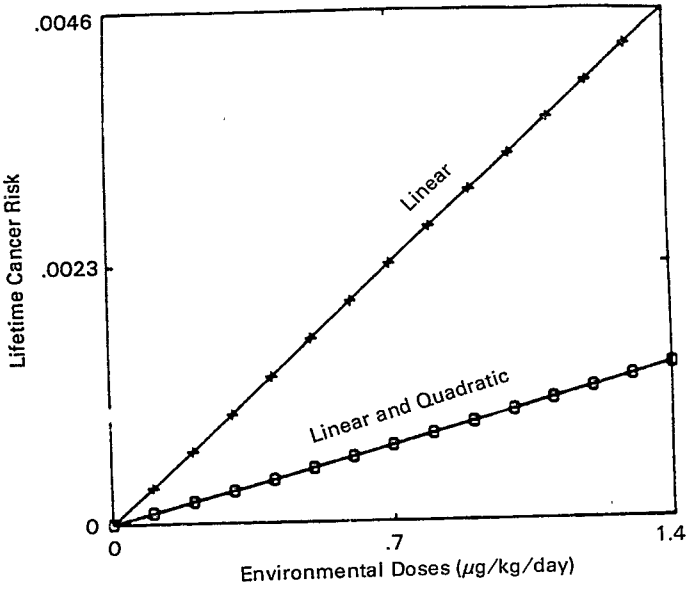

Table B-5. **Results of Model Fitting to Taiwan Skin Cancer Data, Adjusted for Background Rate[a,b]**

Linear	Quadratic
Males:	
Doses (d): 0, 10.818, 29.909, 50.909 μg/kg/day[c]	
$g(d) = (0.351576 \times 10^{-7})d$	$g(d) = (0.106619 \times 10^{-7})d + (0.558064 \times 10^{-9})d^2$
$H(t) = (t - 6.934)^{2.885}$	$H(t) = (t - 6.867)^{2.903}$
ln L = -596.744	ln L = -590.501
Unit risk: 4.0×10^{-3} $(\mu g/kg/day)^{-1}$	Unit risk: 1.6×10^{-3} $(\mu g/kg/day)^{-1}$
Females:	
Doses (d): 0, 6.8, 18.8, 32.0 μg/kg/day[c]	
$g(d) = (0.614891 \times 10^{-8})d$	$g(d) = (0.238789 \times 10^{-9})d^2$
$H(t) = (t - 9.0)^{3.225}$	$H(t) = (t - 9.0)^{3.233}$
ln L = -317.188	ln L = -309.892
Unit risk: 3.0×10^{-3} $(\mu g/kg/day)^{-1}$	Unit risk: Not available due to nonlinearity.

[a]Background rate used is nonmelanoma skin cancer incidence among Singapore Chinese (1968-1977) (IARC, 1976).
[b]Data from Tseng et al., 1968.
[c]Dose estimate for U.S. persons (see Table B-3).

These prevalence rates, in 10-year age categories, were collapsed to form the age groups used in the Taiwan study: < 19, 20 to 39, 40 to 59, and ≥ 60 years. However, since the age distribution of persons over 60 years old differed significantly in the two towns, information on the prevalence of skin cancer in this age group is not included in this analysis.

An evaluation of how well the model, based on the Taiwan experience, predicts the prevalence rates reported by Cebrian et al. (1983) is provided in Table B-6. Since the Mexican prevalence rates are not gender-specific, the Taiwan data for both genders were combined, normalized to dose equivalents in μg/kg/day for the reference U.S. person, and refitted to the model. For the same reason, it was necessary to convert the Mexican dose estimate to that of the reference U.S. person. This was done by assuming that a Mexican male (female) weighs 60 (55) kg and drinks 3.5 (2.5) L of water daily (Cebrian et al., 1983). If there were an equal number of males and females, the reference Mexican person would weigh approximately 57 kg and drink 3 L of water daily. The equivalent dose of arsenic, normalized to the reference U.S. person, appears in Table B-7.

Cebrian et al. (1983) did not report gender difference in susceptibility to skin cancer from arsenic ingestion. There was a significant difference in the Taiwan study, however, where the crude male-to-female ratio was 2.9:1. For this analysis, attempting to ascertain how well the model, using the Taiwan data, might predict the skin cancer response in Mexico, the Taiwan

Table B-6. **Lesions Counted as Skin Cancers (Ulcerative Lesions [UL] and Papular Keratosis [PK]) in Mexico Study, and Predictions Based on Taiwan Experience, Both Genders Combined**

Arsenic concentration (ppm)	Age group (years)			
	0-19	20-39	40-59	≥60
Control town				
UL (observed)	0/201[a] (0)[b]	0/73 (0)	0/29 (0)	0/15 (0)
PK (observed)	0/201 (0)	0/73 (0)	0/29 (0)	0/15 (0)
Exposed town				
UL (observed)	0/187 (0)	1/68 (1.5)	2/27 (7.4)	1/14 (7.1)
PK (observed)	0/187 (0)	8/68 (11.8)	6/27 (22.2)	1/14 (7.1)
UL (predicted)	0.08/187 (0.04)	0.7/68 (1.0)	1.2/27 (4.4)	--

[a]Data from Cebrian et al., 1983.
[b]Prevalence in percentages.

Table B-7. **Conversion of Arsenic Dose for Mexicans to Equivalent Arsenic Dose for U.S. Persons[a]**

Mexican person (ppm)	U.S. person (μg/kg/day)
0.005	0.26
0.411	21.63

[a]Assumptions: A U.S. person weighs 70 kg and drinks 2 L of water daily; a Mexican person weighs 57 kg and drinks 3 L of water daily.

response data for both genders were combined, normalized to dose equivalents for the reference U.S. person, and refitted to the model. The model, with linear and quadratic terms in dose, provides a significantly better fit than that with only a linear term ($p < 0.01$ by the asymptotic likelihood ratio test). The parameter estimates for the combined (i.e., sex-blind) data are:

$$g(d) = (0.564398 \times 10^{-8})d + (0.435613 \times 10^{-9})d^2$$

and

$$H(t) = (t - 8.0)^{3.028}$$

This is virtually a three-stage model (k = 3), with induction time of 8 years (w = 8), and quadratic in dose.

Cebrian et al. (1983) reported that the estimated total dose and overall prevalence of lesions in the Mexican study were similar to those in the Taiwan study, except for skin cancer. As previously stated, Cebrian et al. (1983) separately described papular keratosis and ulcerative lesions that were considered compatible with a clinical diagnosis of epidermoid or basal cell carcinomas, but for which no histologic examination was available. The diagnosis of ulcerative lesions in the Mexican study corresponds to the diagnosis of skin cancer in the Taiwan study.

The equation given above, with 21.63 μg/kg/day as the dose rate for the reference U.S. person (i.e., the dose equivalent to the dose received by the exposed Mexican population) (see Table B-7) predicts the following prevalence of skin cancers by ages 19, 39, and 59, respectively: 0.04%, 0.9%, 4.4% (see Table B-6). The responses observed in the age intervals 0-19, 20-39, and 40-59 in the Mexican study are, respectively: 0.0%, 1.5%, and 7.4%. The differences between the values predicted from the Taiwanese data and those observed in Mexico are negligible in view of the small number at risk in the latter study. Adjustment for background rate of skin cancer in the Mexican study increases the predicted prevalence by a negligible amount.

IV. Use of the German Data to Evaluate Taiwan's Dose-Response Model

In 1984, a follow-up study of former patients who had been treated for skin disorders with Fowler's solution (a solution of arsenic) between 1938 and 1958 was conducted by Fierz (1965). (See II.A.3. for a description of this study.)

The total doses in mL of Fowler's solution and in μg/kg of body weight (assuming a 70-kg body weight) are shown in Table B-8. The crude response is the number of patients with skin cancer (total 21) out of those examined (total 262) by total dose.

The "adjusted" response in Table B-8 (adjusted by isotonic regression) is based on the assumption that the true response rate is monotonically non-decreasing over total dose of Fowler's solution: This assumption is probably not strictly true, since some variables not reported in the study (e.g., treatment regimen) differ among patients, and these differences are likely to affect the response.

A rough comparison between the response rates in the study by Fierz (the "German" study) and the Taiwan study can be made by comparing response rates at equivalent total doses. The total dose (in μg/kg) in the Taiwan study for each dose rate and exposure combination is found by multiplying the daily

Table B-8. **Skin Carcinomas in Patients Treated with Fowler's Solution Who were in the Fierz Follow-Up Study[a]**

Fowler's solution (milliliters)	Crude response	Adjusted response[b]
0 - 50	0/24 (0.0)	0/24 (0.0)
50 - 100	2/45 (4.4)	2/45 (4.4)
100 - 150	2/24 (8.3)	6/98 (6.1)
150 - 200	1/12 (8.3)	6/98 (6.1)
200 - 250	1/14 (7.1)	6/98 (6.1)
250 - 300	1/31 (3.2)	6/98 (6.1)
300 - 350	1/17 (5.9)	6/98 (6.1)
350 - 400	2/11 (18.2)	6/61 (9.8)
400 - 450	2/11 (18.2)	6/61 (9.8)
450 - 500	0/7 (0.0)	6/61 (9.8)
500 - 600	1/18 (5.6)	6/61 (9.8)
600 - 700	1/14 (7.1)	6/61 (9.8)
700 - 1,000	2/13 (15.4)	2/13 (15.4)
1,000 - 1,500	4/15 (26.7)	5/20 (25.0)
1,500	1/5 (20.0)	5/20 (25.0)

[a]Response is given as no. carcinomas/no. patients at risk, and, in parentheses, as a percentage.
[b]Estimate obtained by isotonic regression, assuming true response rates are monotonically non-decreasing as total dose increases.
SOURCE: Fierz, 1965.

dose rate by the total number of exposure days. Assuming an average bodyweight of 70 kg and a weight of 7.6 mg arsenic per mL of Fowler's solution, we multiply the total dose in µg/kg by 9.2×10^{-3} to obtain an estimated equivalent dose in mL of Fowler's solution (FS).[1] The prevalence rate at the resulting total dose in the German study is then read from the adjusted response column in Table B-8.

Exposures in the Taiwan study were far greater than those in the German study. At 10.8 µg/kg/day for 20 years, the total Taiwan dose corresponds to 725 mL. At this dose, the prevalence rate for the Taiwan study is less than 2%. At the equivalent dose in the German study, the prevalence rate is

[1]µg arsenic/kg $\times 10^{-3}$ mg/µg $\times$ 70 kg $\times$ 1/(7.6 mg arsenic/mL FS) = 9.2×10^{-3} mL FS.

estimated to be 15.4% if 262 persons are considered at risk (see Table B-8) and 3.4% if 1,170 are at risk.

Therefore, the difference in the prevalence rates at equivalent total doses estimated from the German study and observed in Taiwan are unknown but may be due to such factors as the difference in dosing regimens and media, the difference in arsenic species in well water in Taiwan and in Fowler's solution, the mitigating effect of other chemicals present in well water, and genetic cultural or socioeconomic differences.

V. Discussion of the Uncertainties of the Risk Estimates

There are several factors that could affect the risk estimates presented in the Special Report. (Some of these factors have already been discussed elsewhere in that document.) In this section, two quantitative issues that received the most comments from peer reviewers are discussed and evaluated.

The first issue concerns the use of prevalence rates to estimate the cumulative incidence rate. As discussed previously, for the prevalence data to be useful for the quantitative risk assessment, three assumptions must be made:

(1) the mortality rate was the same in the diseased (skin cancer) individuals as in the nondiseased individuals.

(2) the population composition (with respect to the risk factors of the skin cancer) remained constant over time.

(3) the skin cancer was not surgically removed.

The appropriateness of these assumptions have been discussed previously in this Appendix. The major concern was that the first assumption may not be appropriate and, thus it is of interest to assess the impact of differential mortality on the risk estimates.

To calculate the age-specific skin cancer rate in the age-interval (x, x + t), the following notations are used:

P_0 = the skin cancer prevalence at age x

P_1 = the skin cancer prevalence at age x + t

m_0 = the mortality rate in the nondiseased persons in the age-interval (x, x + t)

m_1 = the mortality rate in the diseased persons in the age-interval (x, x + t)

h = the age-specific skin cancer rate in the age-interval (x, x + t)

The time to death or skin cancer is assumed to follow the independent exponential distribution with parameters m_i, i = 0, 1, or h. The relationship between the age-specific skin cancer incidence rate, h, and the cumulative incidence, F(t), by time t, is given by

$$F(t) = 1 - exp\,[-\int_0^t h(x)\,dx]$$

Thus, it is sufficient to evaluate the effect of differential mortality on the age-specific incidence.

It is shown (Podgor and Leske, 1986) that the age-specific incidence rate, h, satisfies the following equation.

$$\frac{(1-P_0)P_1 exp(-m_0-h)}{1-P_1} = P_0\, exp(-m_1) +$$

$$\frac{(1-P_0)h[exp(-m_1) - exp(-m_0-h)]}{m_0 - m_1 + h}$$

From this equation, it is possible to investigate the effect of differential mortality on the age-specific skin cancer incidence.

Recall that the risk estimates are calculated under the assumption that those persons with and without skin cancer had the same mortality rate. To assess how an increase of mortality rate in the skin cancer patients can affect the age-specific incidence rate, the skin cancer prevalence rates observed in the Taiwanese males (Table B-1) are taken as an example, and the age-specific skin cancer incidences in various age intervals are calculated using the formula given above. Table B-9 gives the estimated age-specific skin cancer incidence when the relative mortality rates between those persons with and without skin cancer are assumed to be (a) equal ($m_1 = m_0$), (b) two ($m_1 = 2m_0$), and (c) three ($m_1 = 3m_0$).

From Table B-9, it is seen that the age-specific skin cancer incidence assuming differential mortality exceeds those assuming equal mortality, the increase ranging from about 2% to 24% when the relative mortality rate of two ($m_1 = 2m_0$) is assumed; from about 2% to 49% when the relative mortality rate of three ($m_1 = 3m_0$) is assumed. These observations are consistent with Dr. Lin's comments that the difference between the cumulative incidence and the prevalence incidence will be higher in the "high" endemic area than in the "low" endemic area (Lin, 1987).

Since the mortality rate in the diseased (skin cancer) persons is not likely to be three times greater than the nondiseased persons, the extent of risk underestimation does not appear to be of concern.

The second issue concerns the intake of arsenic from the sources other than the drinking water. Arsenic intake from sources other than the drinking water would overestimate the unit arsenic risk calculated above from the Taiwan study. Heydorn (1970) reported that the blood arsenic levels were higher in the Taiwanese than in persons in Denmark, suggesting that both the study and comparison population in the Tseng study may have been exposed to arsenic from sources other than drinking water. However, these data are of limited use because the sample size is small (less than 20) and the sampling protocol is not specified. Since the arsenic-contaminated water was known to be used for vegetable growing and fish farming, the food consumption could have been an important source of arsenic intake in addition to the drinking water. There is very little information on the arsenic content in food, however, that can be used in the risk calculation. To provide some insight about how the arsenic intake from food consumption can affect the risk estimate, the consumption of rice and sweet potatoes is taken as an example.

For the studied population, rice and sweet potatoes were the main staple and might account for as much as 80% of food intake per meal. For the

Table B-9. **Age-Specific Incidence Rates Calculated from Age-Specific Prevalence with Equal and Diffferential Mortalities**

Skin Cancer Age-Specific Incidence[a]

Exposure group[b]	Age	Observed skin cancer prevalence	Equal mortality $m_1 = m_0$	Differential mortality[c] $m_1 = 2m_0$	Differential mortality[c] $m_1 = 3m_0$
Low-dose	20-39	1.07×10^{-3}	1.07×10^{-3}	1.09×10^{-3} (2)	1.11×10^{-3} (4)
	40-59	6.13×10^{-3}	5.94×10^{-3}	6.04×10^{-3} (2)	7.06×10^{-3} (2)
	60-69	4.66×10^{-2}	4.16×10^{-2}	4.84×10^{-2} (16)	5.56×10^{-2} (34)
Mid-dose	20-39	3.77×10^{-3}	3.78×10^{-3}	3.85×10^{-3} (2)	3.91×10^{-3} (3)
	40-49	4.85×10^{-2}	4.59×10^{-2}	5.30×10^{-2} (2)	6.06×10^{-2} (3)
	60-69	1.64×10^{-1}	1.29×10^{-1}	1.57×10^{-1} (22)	1.85×10^{-1} (43)
Low-dose	20-39	2.22×10^{-2}	2.25×10^{-2}	2.29×10^{-2} (2)	2.33×10^{-2} (4)
	40-59	9.89×10^{-2}	8.17×10^{-2}	8.39×10^{-2} (3)	8.61×10^{-2} (5)
	60-69	2.54×10^{-1}	1.89×10^{-1}	2.34×10^{-1} (24)	2.81×10^{-1} (49)

[a]The mortality rates for those without skin cancer are assumed to be 0.035, 0.26, and 0.25 respectively for the age-intervals 20 to 39, 40 to 59, and 60 to 69.
[b]For the low exposure group, $P_0 = 0$, $P_1 = 1.07 \times 10^{-3}$ for the age-interval 20 to 39; $P_0 = 1.07 \times 10^{-3}$; $P_1 = 6.13 \times 10^{-3}$ for the age-interval 40-59; $P_0 = 6.13 \times 10^{-3}$; $P_1 = 4.66 \times 10^{-2}$ for the age-interval 60+ (assumed to be 60 to 69). For other exposure groups, P_0 and P_1 are similarily defined.
[c]The parenthesized values are the ratio (x100) of age-specific skin cancer incidence rates calculated respectively under the assumptions of the differential mortality and equal mortality.

purpose of discussion we will assume that a man in the study population ate one cup of dry rice and two pounds of potatoes per day and that the amount of water required to cook the rice and potatoes was about 1 L. Under this assumption, the risk calculated before is overestimated by about 30% (1 L/3.5 L). This calculation considers only the water used for cooking; the arsenic content in the rice and potatoes that might have been absorbed from soil arsenic is not considered because of the lack of information. For a realistic adjustment of the risk estimates, one would need the information on the arsenic content and the composition of the diet taken by the studied population whose diet content was certainly different from the population currently living in the same area.

VI. Summary

This section presents a dose-response analysis for skin cancer from exposure to inorganic arsenic in drinking water. Results based on the multistage theory of carcinogenesis have been obtained from the Taiwan

epidemiologic study and are compared to two studies in other environments (Mexico: Cebrian et al., 1983; and Germany: Fierz, 1965). Compatibility of results across studies (1) suggests the conclusion that arsenic exposure is the likely causal factor in the increased prevalence of skin cancers in these studies; (2) provides additional statistical evidence for refinement of statistical estimates; and (3) helps to identify potential sources of variability and environmental factors, or patterns of exposure, that may be influential.

None of these studies contains all of the details needed for an ideal statistical analysis, such as: ages at times of initial exposure, termination of exposure, and first appearance of skin cancer; similar information on lesions that may frequently precede appearance of skin cancer; number of subjects with cancer at multiple sites; locations of cancers; and prior disease including those that lead to the use of Fowler's solution. Consequently, it is important to glean what information is available from each study for purposes of complementarity as well as comparison.

Analysis of the Taiwan data required estimation of the number at risk in each dose/age category because only response rates and marginal totals by age groups are provided. The estimated values, which fit the marginal data closely, make possible the estimation of dose-response for the generalized multistage model by means of maximum likelihood. The cancer response is well described by a quadratic polynomial in dose (with positive linear coefficient) for both male and female data. The minimum tumor induction time is estimated at 7 and 9 years for males and females, respectively; in both cases, the cancer response for time-to-tumor is best described by time of observation (minus induction time) to the third power. The observed data in the Mexican study, taken at only one concentration of arsenic in well water, but collected for different exposure intervals, are consistent with predictions from the model using the Taiwan data.

The data from the study in Germany consist of the response of former dermatology patients who had been treated with Fowler's solution (a 0.5% solution of arsenic trioxide, which is a relatively toxic form). Patients were treated for up to 26 years (many for apparently a much shorter period) in intermittent dosing patterns specific to the prescribed treatment. This is in contrast to exposure to arsenic-contaminated well water which is likely to be consumed at a reasonably uniform rate over time.

The published data do not include much information that could be useful for risk assessment. Except for a few specific cases cited here, the data were summarized by response for total dose. When compared to predictions from the model for Taiwan with total dose held fixed at values equivalent to total doses in the German study, and then varied over a wide range of possible exposure durations in the Taiwan data, the skin cancer prevalence values in the German study exceeded the values predicted.

In conclusion, the lifetime risk of skin cancer for a 70-kg person who consumes 2 liters per day of water contaminated with 1 μg/L of arsenic is calculated to range from 3×10^{-5} (on the basis of Taiwanese females) to 7×10^{-5} (on the basis of Taiwanese males); equivalently, the lifetime risk due to 1 μg/kg/day of arsenic intake from water ranges from 1×10^{-3} to 2×10^{-3}.

Appendix C

Internal Cancers Induced by Ingestion Exposure to Arsenic

Internal Cancers Induced by Ingestion Exposure to Arsenic

As noted in the Technical Panel's Special Report on Ingested Inorganic Arsenic, arsenic ingestion has been associated with cancer of internal organs. Chronic arsenic ingestion has been reported to be associated with cancer of the lung (Calnan, 1954; Robson and Jellife, 1963; Fierz, 1965; Chen et al., 1985, 1986), bladder (Sommers and McManus, 1953; Nagy et al., 1980; Chen et al., 1985, 1986), liver (Fierz, 1965; Regelson et al., 1968; Lander et al., 1975; Popper et al., 1978; Roat et al., 1982; Falk et al., 1981; Chen et al., 1985, 1986), nasopharynx (Prystowsky et al., 1978), kidney (Chen et al., 1985; Nurse, 1978), and other internal organs (Rosset, 1958; Reymann et al., 1978; Chen et al., 1985). Many of these references are case reports, however, and do not deserve the attention given a well-designed epidemiologic study.

The Technical Panel felt it important to summarize the studies of Chen et al. (1985, 1986) since these studies have been referred to in the text of the Technical Panel's report, and they are of a design which allows one to give greater weight to observed associations. Chen et al. (1985) calculated cancer standardized mortality ratios (SMRs) for the population of the arsenic endemic area studied by Tseng et al. (1968). The authors found the SMRs for cancer of the kidney, bladder, skin, lung, liver, and colon to be significantly elevated in both males and females. Chen et al. (1986) conducted a case-control study of lung, bladder, and liver cancer mortality cases and randomly sampled controls from the endemic area. They found odd ratios that were significantly ($p < 0.05$) elevated, and remained much the same when adjusting for other risk factors including cigarette smoking. Chen et al. (1985) indicated a positive correlation between the SMRs of those cancers which were significantly elevated and Blackfoot disease prevalence rates. Also, SMRs were greater in villages where only artesian wells were used as the drinking water source than in villages using shallow wells only. Chen et al. (1985) stated that water from the artesian wells in the Blackfoot disease endemic areas had been reported to have from 0.35 to 1.14 ppm arsenic with a median of 0.78 ppm while the shallow well water had arsenic content between 0.00 and 0.30 ppm with a median of 0.04 ppm. Chen et al. (1986) found an increased risk of lung, bladder, and liver cancer with increasing duration of artesian well use. Thus, in both studies (Chen et al., 1985, 1986), the authors demonstrated a qualitative relationship between arsenic exposure and internal cancer risk; however, the data is not sufficient to assess the dose-response. For this purpose, it is necessary to have the individuals studied by Chen grouped by well-water arsenic concentration and age. These data quite likely do (or did) exist, because they were available to Tseng et al. (1968) for the skin cancer study. EPA is currently trying to obtain these data.

Appendix D

Individual Peer Review Comments on Essentiality

Individual Peer Review Comments on Essentiality

This appendix seeks to clarify some uncertainty in the workshop report of the Subcommittee on Essentiality.

The Subcommittee on Essentiality of the December 2-3, 1986 peer review workshop reported that "information from experimental studies with rats, chicks, minipigs, and goats demonstrates the plausibility that arsenic, at least in inorganic form, is an essential nutrient. A mechanism of action has not been identified and, as with other elements, is required to establish fully arsenic essentiality."[1]

The Subcommittee also described a framework for determination of nutritional essentiality. The framework describes the usual approach to establishing essentiality as including:

1) performance of empirical observations in animal models to establish the plausibility of nutritional essentiality;
2) establishment of a reproducible syndrome through the use of chemically defined diets in animal models;
3) definition of biochemical lesions to characterize the specificity of the lesions;
4) establishment of specific biochemical functions absolutely dependent on the factor being investigated.

The Subcommittee's statement on the animal studies clearly addresses points 1 and 4 in the framework, but the written report does not explicitly address points 2 and 3 for the animal studies. Furthermore, Agency participants and some Subcommittee members contacted by telephone differed somewhat in their recollection of the Subcommittee's opinion on the extent to which points 2 and 3 in the above hierarchy had been experimentally achieved. Some selected peer reviewers' comments and observers' notes are summarized below to explain the Technical Panel's position on this issue. The summary report of the Risk Assessment Forum Peer Review Workshop on Arsenic (U.S. EPA, 1987) presents all of the post-workshop comments in full.

I. Comments on Plausibility of Arsenic Essentiality in Animals

A. *Post-Workshop Comments on Essentiality [page numbers refer to the summary report of the Peer Review Workshop on Arsenic (U.S. EPA 1987)]*

Menzel: The section [in the peer review draft] on [essentiality] of arsenic should be rewritten with a more positive emphasis on the probable [essentiality] of arsenic. . . (p. E-17).

[1] Report of the EPA Risk Assessment Forum Peer Review Workshop on Arsenic, December 2-3, 1986.

Mushak: . . .the overall conclusion would seem to be that it is premature to conclude that essentiality is established (p. E-21).

Weiler: It appears that there may be enough experimental evidence to suggest that in some animals, diets low in arsenic affect growth and fertility. However, the levels in the arsenic depleted diet are about the same as those found in the normal human diet (≤50 ng/g). Further, the amount of arsenic added as a supplement (2 µg/g) are far in excess of what would be found in the normal human diet.

Further, the supplementary arsenic is all inorganic, whereas the arsenic in the human diet is, in all likelihood, almost all organic. Thus, the amount of inorganic arsenic in the human diet (excluding drinking water) is really quite small (perhaps a few µg/day), but there are no apparent health effects that have been observed in humans. The relevance of the animal experiments to humans is therefore not at all clear and it seems unrealistic to believe that arsenic is needed in quantities greater than what is present in the normal western diet (pp. E-43 through E-44)

B. *Oral Comments Drawn from EPA Notes of Meeting:*

- The absence of knowledge of biochemical action for arsenic and of cofactor requirements renders a determination of essentiality uncertain (methyl donors, vitamin C, choline, molybdenum, arginine, and histidine were cited as possible cofactors). [Fox; Combs; general [2]]
- Reproductive experiments are difficult to perform and not always reproducible. Discussants referred again to lack of knowledge of possible cofactors. [Nielsen; Menzel; general]
- Progression of steps leading to the establishment of essentiality is necessary. Several participants felt that research is now in an early stage (i.e., step 2, establishment of a reproducible syndrome). [Combs; general]
- Some reviewers emphasized that the steps in the framework need not all be unambiguously established, e.g., identification of a specific biochemical lesion and mechanism would suffice even in the absence of a clear definition of a reproducible syndrome. [general]

II. Estimation of a Human Nutritional Requirement for Arsenic

The Subcommittee's report states ". . .at this time it is only possible to make a general approximation of amounts of arsenic that may have nutritional significance for humans."[3]

[2] General discussion. Individual attribution uncertain.

[3] Report of the EPA Risk Assessment Forum Peer Review Workshop on Arsenic, December 2-3, 1986.

A. *Post-Workshop Comments [page numbers refer to the summary report of the Peer Review Workshop on Arsenic (U.S. EPA 1987)]*

Menzel: . . .the development of the estimate for the human daily requirement is quite limited and careful delineation of the limits should be included. . . .uncomfortable about providing a single estimate and would encourage the provision of a range of values citing the uncertainties in the methods of estimation and the interactions between arsenic and methyl donor. . .availability in the diet (p. E-17).

Strayer: I feel that a certain tone could be struck by the report to indicate that evaluating the question of lower limits for arsenic in drinking water is not so much a matter of direct proof of essentiality in any species. Rather, the fact that the possibility of essentiality has been raised by workers in widely disparate species and settings should deter us from setting very low limits even if proof of its essentiality in man is not forthcoming (p. E-30).

B. *Oral Comments Drawn from Observers Notes of Meeting*

- Discussants outlined reasons for not providing an estimate of nutritional requirements for arsenic at this time: the fact that there is no information on speciation of arsenic in the diet; analytical difficulties; species-comparative problems (e.g., uncertainty on whether to make direct weight comparisons or to use surface area conversions); lack of a biochemical mechanism; and lack of knowledge of arsenic requirements as a function of age. [general]

- Discussants reached a consensus that development of an order-of-magnitude estimate of intake requirements is possible. However, they felt that the factors influencing the uncertainty of such an assessment (as listed above) should be spelled out. [general; subcommittee report [4]]

III. Use in Risk Assessments

Andelman: At the workshop it was the consensus that the essentiality of arsenic has not been proven for humans. . . .Nevertheless, there does seem to be some confusion in that the question of essentiality has become somewhat intertwined with that of the risk for skin cancer, and this is inappropriate. The risk of skin cancer is unlikely to be influenced by the *possible* essentiality of arsenic. The use of the risk model to regulate arsenic should take into account such a possibility, but there does not appear to be a basis for doing so at this time (p. E-6).

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[4] Report of the EPA Risk Assessment Forum Peer Review Workshop on Arsenic, December 2-3, 1986.

Menzel: As a consequence of the agreement of the workshop participants on the probable essentiality of arsenic, a new section will have to be added to deal with [the] problem [of essentiality versus toxicity]. . . .EPA should face . . .the problem of the no-threshold treatment of oncogenesis and the threshold phenomenon of essentiality. . . .

I see no need to abandon the no-threshold treatment for oncogenesis even though arsenic or other minerals might be essential. To not face this issue directly will only encourage misunderstanding and disagreement with the risk estimate (pp. E-18 through E-19).

Mushak: It is premature to factor essentiality into risk assessment models for arsenic exposure in human populations. . . .There is no inherent limitation on the use of linear extrapolation models for, e.g., skin cancer, because of any threshold implicit in a daily required intake (p. E-21).

Appendix E

Metabolic Considerations

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Tables

Figures

I. Introduction

The Technical Panel has concluded that ingestion of inorganic arsenic can produce a dose-related carcinogenic response in humans. There are many uncertainties including the mechanism of action of arsenic as a human carcinogen. The Technical Panel has explored the bioavailability, toxicity, and carcinogenicity of the different chemical forms of arsenic which comprise the U.S. body burden and outlined this information in broad overview in this Appendix. However, the Panel expects that EPA program offices will use their own information developed for particular conditions of human exposure, along with the information presented in this Appendix, to develop a complete risk assessment for this compound.

This Appendix also delineates the metabolic pathways of absorption and the daily ingested amount of arsenic at which excretion and elimination of arsenic occur. The many new studies available on arsenic metabolism may offer explanations for some of the observations reported in the epidemiologic studies, provide a basis for speculation about the role of some of these metabolic factors in the carcinogenesis of arsenic, and suggest avenues for future research. Although much of the data on pharmacokinetics is derived from acute or short-term exposures, a number of observations are cited of populations chronically exposed occupationally or through drinking water and food. However, the Panel remains uncertain about the applicability of this information *in toto* to carcinogenesis developing under conditions of chronic exposure. The Panel believes, however, that information and analyses of this type will be useful in future assessments of the risks associated with human exposure to arsenic.

Part III reviews information on sources of arsenic to provide data on the body burden of arsenic in the U.S. population. In Part III data relating to the metabolism and toxicity of arsenic are reviewed as background for the discussion in Part IV of metabolic considerations that may help elucidate the mechanism by which arsenic effects carcinogenic changes in humans.

II. Exposure Levels of Arsenic; Chemical Forms and Availability

Arsenic is a natural constituent of certain rock and mineral formations in the earth's crust. Weathering of rocks and minerals appears to be a major source of arsenic found in soils and drinking water sources. Other causes of arsenic in soil are deposition and precipitation of airborne particles from industrial operations, application of arsenic-containing pesticides, and decay of contaminated plant material. As a result of its ubiquitous nature, humans are exposed to arsenic primarily in foodstuffs and drinking water, and for certain target groups, from industrial and agricultural uses (U.S. EPA, 1985). Among individuals of the general population, the main routes of exposure to arsenic are via ingestion of food and water; lesser exposures occur via inhalation. Among smokers, intake by inhalation is augmented in proportion to the level of smoking because of background levels of arsenic in tobacco (Weiler, 1987; IARC, 1986).

A. *Drinking Water*

Drinking water contains arsenic predominantly as inorganic salts in the trivalent and pentavalent states. These inorganic salts are fully available biologically and quite toxic in very high concentrations. In chlorinated drinking water supplies, all arsenic salts have been found to be pentavalent as a result of oxidation by free chlorine.

The results of federal surveys of public water supplies and compliance monitoring data developed by the states are summarized below (U.S. EPA, 1984b; U.S. EPA, 1985). Most of the approximately 214 million people in the United States using public water supplies are exposed to levels of arsenic below 2.5 µg/L. Assuming an average daily consumption of 2 liters of water, most of the U.S. population would thus be exposed to less that 5 µg of arsenic per day from drinking water. However, some U.S. drinking water supplies contain higher concentrations of arsenic. Based on the compliance monitoring data available through the Federal Reporting Data Systems, one can estimate that approximately 112,000 people are receiving drinking water from public water supplies with arsenic levels at or above 50 µg/L, the current Maximum Contaminant Level. These people would be exposed to more than 100 µg of arsenic per day. These surveys do not include many wells currently in use in the United States. On the average, ground water supplies show higher levels of arsenic in some of the western United States.

B. *Ambient Air*

Assuming a daily inhalation rate of 20 m^3, and an average national exposure of 0.006 µg arsenic/m^3, the inhalation exposure of the general public to water-soluble forms of arsenic in ambient air can be estimated as almost 0.12 µg/day. Assuming 30% to 85% absorption of inhaled arsenic, depending on the relative proportions of vapor and particulate matter (U.S. EPA, 1984a; Vahter, 1983), the general public would be exposed to a range of approximately 0.04 to 0.09 µg/day of arsenic by inhalation.

Persons living near industrial areas such as smelters, glass factories, chemical plants, or cotton gins may be exposed to ambient air levels between 0.1 and 3.0 µg arsenic/m^3 (U.S. EPA, 1984b). This would result in as much as 45 µg arsenic absorbed per day.

In the general environment, airborne arsenic is available from a variety of sources as inorganic salts. In the vicinity of smelters, these salts contain trivalent arsenic. The chemical form and the uptake rate of arsenic in the vicinity of cotton gins from its use as a desiccant on cotton is not known.

C. *Food*

In the United States, arsenic is used as a pesticide on grapefruit, grapes, and cotton. In addition, the animal feed use of cotton, grapes, and grapefruit byproducts can lead to arsenic residues in meat and milk. Various organic forms of arsenic (arsanilic acid, roxarsone, and carbarsone) are added to feed as growth enhancers for chickens and swine (Anderson, 1983). Finally, many food-stuffs contain arsenic from background environmental contamination.

Food arsenic values taken from FDA surveys indicate an average daily dietary intake of approximately 50 µg arsenic (Johnson et al., 1984; Gartrell et al., 1985; U.S. EPA, 1984 a,b). Generally, the meat, fish, and poultry composite group is the predominant source of arsenic intake for adults and has been estimated to account for about 80% of arsenic intake (Gartrell et al., 1985; Hummel, 1986; 1987; U.S. EPA, 1984b). Of this composite group, fish

and seafood consistently contain the highest concentrations of arsenic. The concentration of arsenic in fish and seafood (particularly shell fish and marine foods) is generally one to two orders of magnitude higher than that in other foods (FDA, 1985; Jelinek and Corneliussen, 1977). The second most concentrated source of arsenic in these FDA surveys is the grain and cereal group which may account for about 17% of arsenic. Following these groups are vegetables, sugars, oils, fats, and beverages. In the average U.S. adult diet, dairy products account for 26% by weight; meat, fish, and poultry 9%; grain and cereal products 14%; potatoes 5%; fruits 11%; and vegetables 6% (Gartrell et al., 1985).

An analysis of arsenic species in foods sampled by the Canadian government shows that most of the arsenic in meats, poultry, dairy products, and cereals is inorganic (Weiler, 1987). Fruits, vegetables, and fish contain arsenic predominantly in organic forms. These data, though based on a limited number of samples, are included here (Table E-1) because, until recently, this type of breakdown by arsenic species has not been available.

Table E-1. **Percentage of Inorganic Arsenic in Food: A Preliminary Analysis[a]**

Food	Percentage of Inorganic Arsenic
Milk and dairy products	75
Meat - beef and pork	75
Poultry	65
Fish - saltwater	0
- freshwater	10
Cereals	65
Rice	35
Vegetables	5
Potatoes	10
Fruits	10

[a]Speciation of the arsenic content of basic food groups based on preliminary data from the Ontario Research Foundation and other sources.
SOURCE: Weiler, 1987.

Because of the very large quantities of arsenic in fish and seafood, many investigators have studied the chemical forms of arsenic in fish and their metabolism, excretion, and toxicity in humans. As noted in Table E-1, arsenic in seafood is predominantly organic. A number of researchers have shown that these organic forms are trimethylated. In 1977, Edmonds et al. showed that rock lobster contained 26 ppm of arsenic as arsenobetaine, $(CH_3)_3\ As^+CH_2\ CO_2$. Other researchers have shown that trimethyl arsenic in fish also occurs in other chemical structures, such as arsenocholine.

Yamauchi and Yamamura (1984) showed that although most of the trimethyl arsenic compounds in prawns were excreted unchanged, 3% to 5% is changed to mono- and dimethylated forms or to inorganic arsenic. Thus, although most of the organic arsenic in seafood is excreted rapidly and unchanged, some of it may be retained in the soft tissues, undergo biotransformation, and be available biologically.

D. *Occupationally Exposed Groups*

Pesticide applicators and workers in copper, lead, and zinc smelters, glass manufacturing plants, chemical plants, wood preserving plants, and cotton gins are exposed to high levels of arsenic. Smelter workers are exposed to trivalent arsenic, workers in wood preserving plants are exposed to pentavalent arsenic, and pesticide applicators are exposed to various inorganic salts as well as mono-methyl arsenic (MMA) and cacodylic acid or dimethyl arsenic (DMA).

The OSHA standard is 10 µg arsenic/m^3 (8-hour time-weighted average) for industrial exposure (OSHA, 1986). Using the previous assumption for daily ventilation rate and lung absorption and assuming an 8-hour workday, an occupationally exposed person could receive about 80 µg corresponding to 68 µg water-soluble arsenic absorbed daily via inhalation at the OSHA standard. Because arsenic is poorly absorbed dermally (approximately 0.1%), dermal exposure has been considered to be negligible as compared to inhalation exposure.

E. *Total Daily Body Burden*

Table E-2 represents the range of total body burden of arsenic from all sources: dietary, drinking water, smoking, ambient air, and occupational exposure, in the United States, namely 55.09 to 224 µg/day. As noted in this section, water and air generally contain arsenic in inorganic and organic forms. Using information about the percentages of inorganic arsenic in various food groups, combined with FDA surveillance data on the contributions of these foods to the daily arsenic intake, it appears that the diet including drinking water and beverages contains about 17 or 18 µg/day of inorganic arsenic (Table E-2).

III. Metabolism, Bioavailability, and Toxicity

A. *Toxicity of Arsenic Chemical Species*

Chronic arsenic intoxication can lead to gastrointestinal disturbances, hyperpigmentation, and peripheral neuropathy (Goyer, 1986). Arsenic is also carcinogenic, and Jacobson-Kram (1986) notes that arsenic is clastogenic and causes sister chromatic exchange.

The toxicity of arsenic is closely related to its chemical form. Inorganic salts and acids of arsenic occur predominantly in the tri- and pentavalent oxidation states. It is well known from acute exposure studies that trivalent arsenic is more toxic than pentavalent arsenic (Goyer, 1986). Recent studies have shown that at environmental levels, pentavalent arsenic is rapidly converted to trivalent arsenic in the blood (Marafante et al., 1985). These two forms can be readily interconverted in mammals. Trivalent and pentavalent arsenic salts also have different modes of toxic action. Cellular mechanisms of arsenic toxicity have been discussed in several current reviews (Goyer, 1986; Vahter and Marafante, 1983). For example, Vahter and Marafante note that "Arsenite is known to react with SH-groups of proteins and enzymes

Table E-2. Daily Arsenic Body Burden (μg/day) in the United States

Source	Usual	Unusual
Water	5	100[a]
Air	0.09	1.5 - 45[b] 68[c]
Food	50[d]	50
Smoking		2 - 6[e]
TOTAL	55.09	up to 224

[a]At the ODW maximum containment level (see Part II.A).
[b]Near industrial use sites such as smelter or cotton gins (see Part II.B).
[c]Occupational exposure.
[d]See Part II.C.
[e]2 μg arsenic/package (Weiler, 1987; IARC, 1986).

while arsenate may interfere with phosphorylation reactions due to its chemical similarity with phosphate."

Methylation of inorganic salts of arsenic through the trivalent state appears to be a detoxification pathway in mammals (Vahter, 1983). The simple methylated forms of arsenic, namely cacodylic acid and methanearsonate, are less acutely toxic than the inorganic salts. Fairchild et al. (1977) gives the LD_{50} of arsenic trioxide as 1.43 mg/kg, of MMA as 50 mg/kg, and of DMA as 500 mg/kg. Trimethylated forms of arsenic are not acutely toxic and are rapidly excreted (Vahter, 1983). Although tested in animals, the oncogenic potential of the organic forms has not been adequately characterized.

B. Absorption, Distribution, and Elimination

Arsenic exposure occurs predominantly through ingestion and inhalation. Dermal absorption is negligible. A detailed understanding of the mammalian distribution, elimination, and long-term deposition patterns following exposure and the relationship of these processes to the internal body burden can provide insights into tissue sites for chronic target organ toxicity.

In smelters, inhaled arsenic and that brought to the gastrointestinal tract by mucociliary clearance, leads to approximately 80% absorption (Pershagen and Vahter, 1979). Smith et al. (1977) showed that nonrespirable particulate forms of arsenic were more closely correlated with excretion of arsenic than respirable forms. These results imply that ingested forms of arsenic are better absorbed and get into the bloodstream more efficiently than inhaled arsenic. Marafante and Vahter (1987) compared absorption and tissue retention of arsenic salts administered orally and intratracheally in the hamster. In general, orally administered arsenic had a shorter biological half-life than that administered intratracheally. Clearance of arsenic compounds from the lungs was also closely correlated with solubility under physiological conditions.

Brune et al. (1980) collected autopsy specimens from a group of 21 Swedish smelter workers employed between 10 and 30 years in a smelter. A control group consisted of eight individuals from a region 50 km from the smelter site. Arsenic levels in kidney and liver were comparable for workers and control subjects, but levels of arsenic in lung tissue were about 6 times higher for the smelter workers than the control group. Furthermore, arsenic levels in the lungs of workers retired up to 19 years were comparable to those in workers autopsied less then 2 years after retirement. However, if smoking is a factor, the high lung levels in some subjects may be a function of chronic exposure to arsenic in tobacco smoke. For example, Vahter (1986) reports that some smokers in the 1950s may have inhaled as much as 0.1 µg arsenic each day. Although the complete smoking history of these workers is not known and the duration of exposure of the two groups of retirees is not completely defined, the Brune et al. study may indicate that a portion of inhaled arsenic binds irreversibly to lung tissue.

Valentine et al. (1979) measured arsenic levels in human blood, urine, and hair in five United States communities with arsenic concentrations in drinking water ranging from 6 µg/L to 393 µg/L. Their results showed that arsenic concentrations increased in urine and hair samples in proportion to increases in concentrations in drinking water. However, this trend was not reflected in blood until drinking water concentrations exceeded 100 µg/L.

Various researchers have monitored arsenic excretion in the urine and the feces and found that the urinary tract is the major route of elimination and accounts for more than 75% of absorbed arsenic over time. Animal studies have also shown that little, if any, absorbed arsenic is exhaled (WHO, 1981). Thus, since the late 1970s, pharmacokinetic and metabolism studies have monitored the urine alone as an approximate surrogate for excretion. When organic arsenic is administered orally, it is eliminated more rapidly than inorganic forms. In addition to urine and feces, arsenic is also eliminated from the body via sweating and desquamation of the skin. In humans not excessively exposed to inorganic arsenic, the highest tissue concentration of arsenic is generally found in skin, hair, and nails (Liebscher and Smith, 1968). Kagey et al. (1977) also studied women in the United States and showed that umbilical cord levels of arsenic were similar to maternal levels.

Because of the limitations of human studies of absorption, elimination, and tissue distribution of arsenic, various researchers have used the recent advances in arsenic speciation methods to study the way laboratory animals handle arsenic. Lindgren et al. (1982) injected mice with radiolabeled (inorganic) arsenic and used whole body radiography to study its distribution and clearance. Initial concentrations were highest in the bile and kidney for arsenate, but clearance from these tissues was extremely rapid. After 72 hours, the highest concentrations were in the epididymus, hair, skin, and stomach for arsenite and the skeleton, stomach, kidneys, and epididymus for arsenate. Arsenate was cleared more rapidly than arsenite from all soft tissues but the kidneys. It seems probable that this pattern of uptake is related to the chemical similarities between arsenate and phosphate in the apatite crystals in bone. One can ascribe the accumulation of arsenic in skin, hair, and upper gastrointestinal tract to its binding of sulfhydryl groups of keratin (Goyer, 1986).

Following intravenous injection of DMA in rabbits or mice, excretion was essentially complete within 24 hours, indicating low affinity for the tissues *in vivo* (Vahter and Marafante, 1983). The same results were obtained following oral administration (Vahter et al., 1984). In addition, the distribution showed a different pattern from that shown after administration of inorganic arsenic, as

discussed above. The highest initial concentration of arsenic in mice was found in the kidneys, lungs, gastrointestinal tract, and testes. Tissues showing the longest retention time were the lungs, thyroid, intestinal walls, and lens.

Tissue retention of arsenic in the marmoset monkey, which doesn't methylate arsenic, was much more pronounced than in species which methylate arsenic (Vahter and Marafante, 1985). Seventy-two hours after injection with inorganic arsenic, almost 60% was still bound to the tissues. The major single binding site was liver, with 10% of the original dose. Arsenic was also retained in the kidney and gastrointestinal tract. To the extent that the marmoset monkey may be an appropriate model of distribution and tissue retention in humans when arsenic levels exceed the normal detoxification capacity, these studies may enable us to predict accumulation of arsenic in the liver, kidney, and gastrointestinal tract from chronic high exposure.

In summary, systematic animal studies and observations in humans show that arsenic is efficiently absorbed through the gastrointestinal tract and via inhalation and eliminated predominantly in the urine. High levels of exposure can lead to deposition in tissues rich in sulfhydryl (SH) groups such as the lung tissue, gastrointestinal tract, skin, and hair. Arsenic also appears to concentrate in the liver and to a lesser extent the kidney, especially in the marmoset monkey which does not methylate arsenic. As discussed above, the chemical form of arsenic influences its retention time and target tissue sites.

C. *Detoxification Via Methylation*

Methylation of inorganic arsenic is generally accepted as a detoxification mechanism of mammals. Vahter (1983) and Vahter et al. (1984) showed that methylated arsenic is excreted more rapidly after ingestion than the inorganic forms. In addition, cumulative observations of humans acutely exposed to inorganic arsenic show that, although inorganic arsenic is the predominant initial metabolite, after 9 days, MMA and DMA account for more than 95% of total arsenic excreted in the urine (Mahieu et al., 1981). Various researchers have shown that methylation of inorganic arsenic occurs enzymatically prior to elimination in the urine. The enzymatic pathways for arsenic methylation and detoxification are summarized in this section.

Methylation appears to take place through the trivalent As (+3) state (Vahter and Envall, 1983). Based on studies with model compounds, Cullen et al. (1984) hypothesized that methylation of arsenic III requires s-adenosylmethionine in excess, dithiolipoic acid-like structures on the membranes, and/or a functional enzyme system (see Figures E-1 and E-2).

The major site of methylation appears to be the liver (Klaassen, 1974). Lerman et al. (1985) followed methylation of tri- and pentavalent arsenic in cultures of hepatocytes. They found that dimethyl arsenic acid formed when arsenite, but not arsenate, was added to the culture medium. No metabolism of arsenate was seen, nor was the arsenate taken up by the liver cells. The authors postulated that the differences in *in vitro* cellular uptake of the two forms of arsenic may be due to the fact that, at physiologic pH, arsenite is not ionized, whereas arsenate is charged.

In order to understand reaction mechanisms and sequences of methylation, Buchet and Lauwerys (1985) performed *in vitro* incubations of inorganic arsenic with various (rat) tissues. The methylating capacity of red blood cells, and brain, lung, intestine, and kidney homogenates were insignificant by comparison to that of the liver. They found that the cytosol was the sole fraction of the liver showing methylating activity; and s-

Figure E-1. **Reproduction of arsenic III forms by membrane-bound lypoic acid. Source: Cullen et al., 1984.**

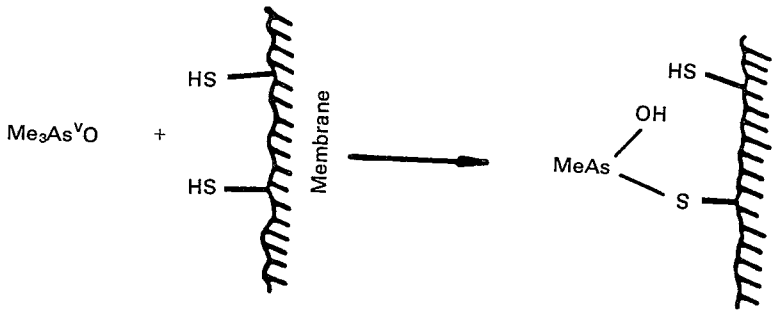
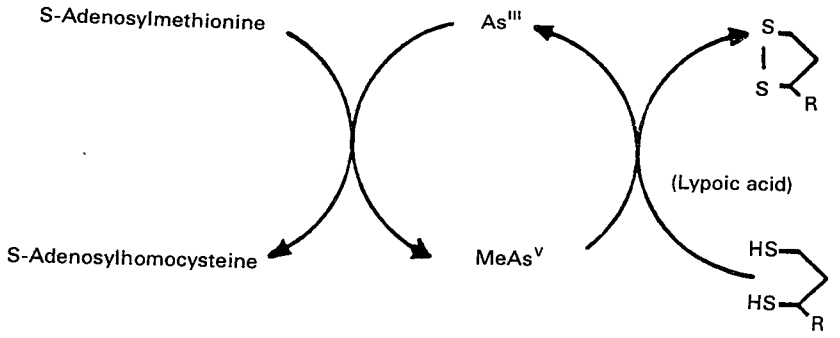


Figure E-2. **Role of s-adenosylmethionine in methylation of arsenic III. Source: Cullen et al., 1984.**



adenosylethionine and reduced glutathione were required as methyl donors. The effect was further enhanced by addition of vitamin B_{12} to this system. Although MMA was formed immediately, a 30-minute latency period occurred before DMA was produced, suggesting that it is formed from MMA. As cytosol and subtrate (As +3) concentrations were varied, MMA and DMA appeared to exhibit different kinetics of formation. At high substrate concentrations, DMA formation was inhibited, while MMA appeared to

accumulate in the system, showing that formation of DMA is a rate-limiting step.

Methyl transferase activity has been shown to play a necessary role in the methylation of arsenic in mammals (Marafante and Vahter, 1984, 1986; Marafante et al., 1985). The effect of dietary deficiencies and genetic variability on methylating capacity (shown below) has important implications for tissue distribution and individual susceptibility to arsenic toxicity.

Marafante and Vahter (1984) studied the effect of methyl transferase inhibition on the metabolism and tissue retention of arsenite in mice and rabbits. Periodate-oxidized adenosine (PAD), an inhibitor of methyl transferase, was injected into mice and rabbits prior to administration of the arsenite. This led to a marked decrease in production of cacodylic acid, a dimethylated form of arsenic. Moreover, impairment of methylation increased the tissue retention of arsenic. These results imply that S-adenosyl-methionine is a methyl donor in the methylation of inorganic arsenic *in vivo* and are consistent with the conclusions of Buchet and Lauwerys (1985) regarding the significance of various cofactors *in vitro*.

In 1985, Marafante et al. measured blood as well as urinary concentrations of arsenic metabolites following the administration of arsenate. The reduction of arsenate to arsenite occurred almost immediately, followed by the appearance of DMA in the blood plasma after about an hour. The administration of PAD led to a dramatic decrease in the appearance of DMA in the blood and confirmed the earlier results in the laboratory showing the significance of methyl transferase activity in the methylative metabolism of arsenic. Urinary excretion of arsenate and its metabolites paralleled their concentrations in the blood. In light of these observations, these authors postulated that reduction of arsenate to arsenite is an initial and independent reaction in the biotransformation of arsenate and probably occurs in the blood.

In a later study, Marafante and Vahter (1986) studied the effect of choline-deficient diets on the metabolism of arsenic in rabbits. Shivapurkar and Poirier (1983) had previously demonstrated that choline- or protein-deficient diets increase relative hepatic concentrations of s-adenosylhomocysteine, leading to inhibition of methyl transferase activity. In their study, Marafante and Vahter showed that both the choline-deficient diets and the administration of PAD led to decreased excretion of DMA in the urine and higher retention of ^{74}As in the liver, lungs, and skin. (As noted above, this pattern is seen in the marmoset monkey which lacks the genetic capacity to methylate arsenic.) In addition, choline deficiencies led to an increased concentration of ^{74}As in the liver microsomes.

These observations demonstrate that methylation as a detoxification pathway is enzymatic and occurs via the trivalent state of arsenic to MMA and subsequently to DMA. Furthermore, decreased methylating capacity caused by chemical inhibition, dietary deprivation, or genetic disposition appears to lead to decreased excretion of DMA in the urine, with retention of arsenic in the lungs, skin, and liver. In addition, certain dietary deficiencies lead to concentration of arsenic in the liver microsomes. These results in animals may be considered to mimic that segment of the human population described as poor methylators. [See the following section for a summary of the human studies by Foa et al. (1984) and Buchet et al. (1982).] They may also serve as models for those populations consuming protein-deficient diets while exposed to high levels of arsenic. In these populations, one can anticipate that decreased methylating capacity can lead to an increased deposition of

arsenic in liver and lung cells as well as the organ sites of normal distribution, namely skin, hair, and nails.

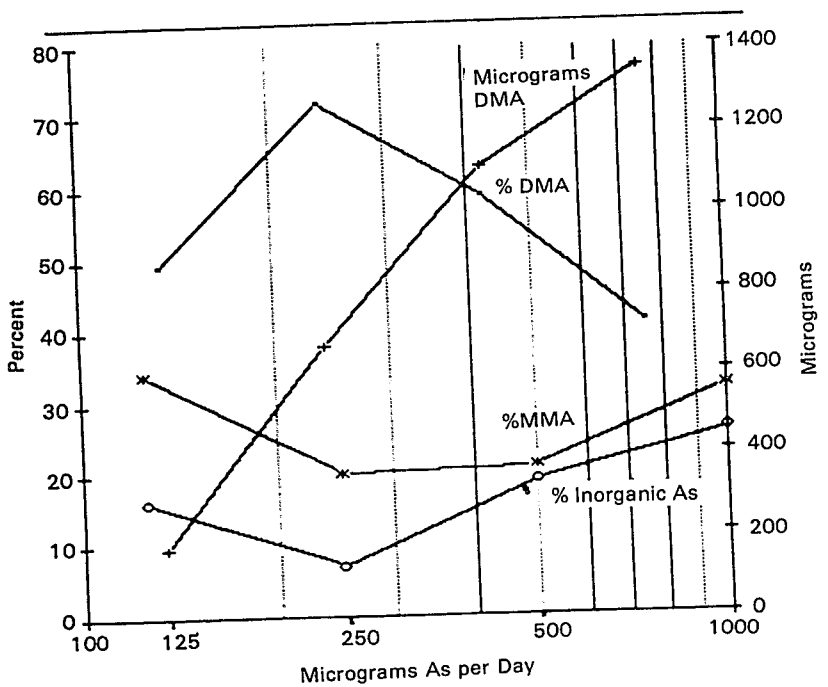
D. Human Metabolism and Enzyme Kinetics

This section contains summaries of human studies of the metabolism and enzyme kinetics of arsenic. In these studies, dosing or exposure levels ranged from background levels to which the general population is normally exposed, through levels representing occupational exposure, up to highly toxic levels. The dosing patterns include acute, short-term, and chronic exposure. Of necessity, many of these studies are limited to single doses in small numbers of human volunteers. Nonetheless, when seen in the context of the enzyme kinetics of arsenic methylation described previously, they provide valuable insights into the way humans can handle, detoxify, and eliminate arsenic at levels of concern.

Buchet et al. (1981) performed a series of pharmacokinetic studies of arsenic metabolism in human volunteers exposed to levels of arsenic roughly comparable to those in smelters. In the first study, groups of three, four, or five adult males drank solutions containing 500 µg equivalents of inorganic arsenic, MMA, or DMA. After a single dose, urine was collected for four days and analyzed for inorganic arsenic, MMA, or DMA. In four days, total or cumulative arsenic content as monitored by urinary excretion, amounted to about 47% of the ingested dose of inorganic arsenic, 78% of ingested MMA, and 75% of ingested DMA, indicating much more rapid excretion of organic than inorganic forms. After ingestion of inorganic arsenic, the percentage of inorganic arsenic excreted in the urine fell off extremely rapidly and was accompanied by an increase of DMA excretion. However, MMA excretion initially increased and then at 12 to 24 hours began to decrease. When MMA was ingested, MMA accounted for 87.4% and DMA accounted for 12.6% of urinary arsenic after 4 days indicating some bioconversion of MMA to DMA, but no demethylation. When DMA was ingested, all urinary arsenic was excreted as DMA. These observations, in light of the relative toxicities of the metabolites, demonstrate that methylation is an efficient detoxification pathway for arsenic.

In a second human study, Buchet et al. (1982) studied urinary metabolites after repeated oral dosing for 5 days with 125, 250, 500, or 1,000 µg inorganic arsenic. In this study, urinary monitoring was performed for 9 days following the last dose. Although only one volunteer was tested at each dose, they were chosen in the context of previous studies in the laboratory to have normal methylation rates. Above 500 µg the ratio of DMA to MMA decreased and methylating capacity appeared to fall off as shown in Figure E-3. When the percentage of each metabolite was plotted against the log of the ingested dose, the concentration (percentage) of inorganic arsenic declined and that of DMA increased commensurate with first-order kinetics. The rate of conversion to methylated forms diminished starting at 250 µg, but not until the dose range exceeded 500 µg did the absolute amount of DMA decline indicating saturation of methylating capacity. In addition, the biological half-life of total recovered arsenic increased with increasing dose (39 h at 125 µg to 59 h at 1000 µg). The authors indicated that when they saw these results, they re-examined the history of the high-dose volunteer, but confirmed that his excretion pattern for arsenic was not out of line with the others. These results suggest the hypothesis that saturation of methylating capacity occurs just above 500 µg/day in healthy adult males exposed to repeated doses of arsenic in short-term experiments. However, confirmation of the enzyme

Figure E-3. Urinary concentrations of arsenic and its metabolites. Source: Adapted from Buchet et al., 1982.



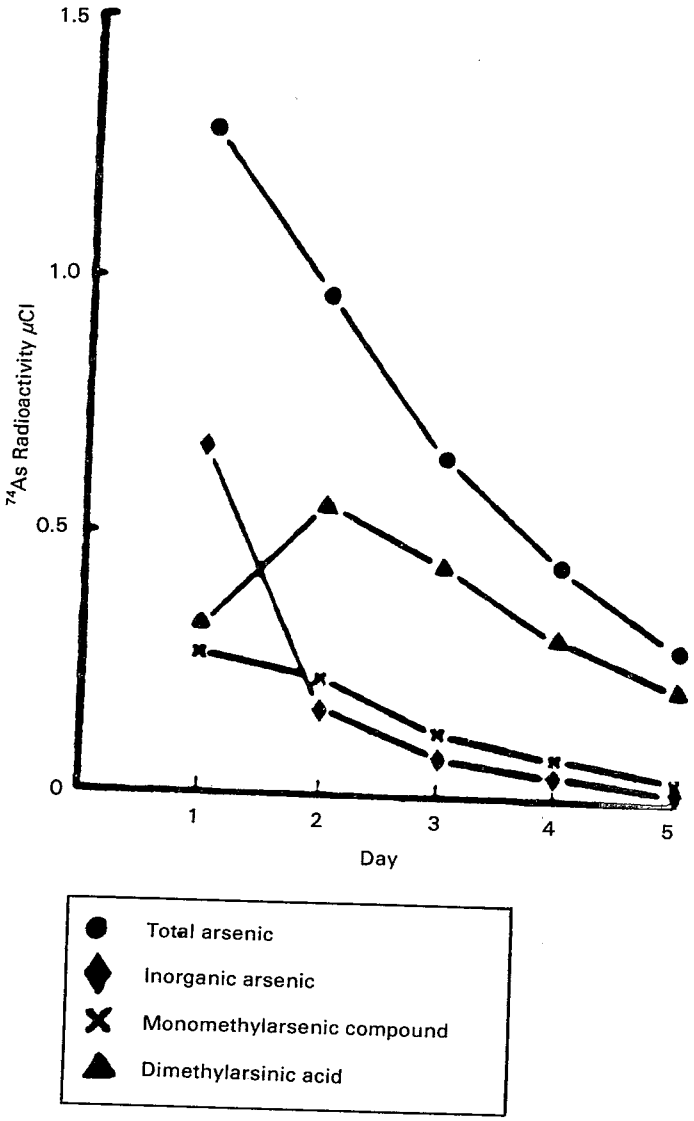
saturation pattern would require that EPA obtain the raw data from Buchet's experiments.

These short-term dose-response curves are typical of enzymatic conversion processes. Buchet's studies include a dosing range up through enzymatic saturation and beyond it. At about 600 µg/day the absolute amount of MMA begins to plateau, and the saturation of methylation occurs between doses of 500 and 1,000 µg/day in people of adequate methylating capacity (Figure E-3).

In 1985, Lovell and Farmer monitored urine for arsenic metabolites following ingestion of highly toxic doses of inorganic arsenic by people attempting suicide. In the course of 5 days, a decreasing percentage of inorganic arsenic was eliminated with a corresponding increasing percentage of DMA, implying metabolic conversion of one to the other. The amount of MMA in the urine did not show any such clear pattern. A similar pattern of urinary metabolites to that observed by Lovell and Farmer (1985) as well as Buchet et al. (1981) was seen by Tam et al. (1979) (Figure E-4).

From the dose-response experiments and the time course of elimination, one can postulate that after the initial rapid excretion of inorganic arsenic arising from ingestion of inorganic arsenic, simple enzymatic conversion to

Figure E-4. Excretion of arsenic metabolites following a single oral dose of inorganic arsenic. ^{74}As radioactivity in urine of male volunteer No. 5; ingested dose: 6.45 μCi.
Source: Tam et al., 1979.



DMA, first order in the inorganic arsenic substrate, occurs in the liver. The DMA is then excreted via the kidneys. However, conversion of arsenic to MMA as observed by urinary excretion does not indicate simple kinetics. Possibly, this conversion occurs at the cellular level throughout the body, or by nonenzymatic mechanisms. In light of this elimination pattern for short-term experiments, conversion of inorganic arsenic to DMA appears to be the rate-limiting step in detoxification (Buchet and Lauwerys, 1985).

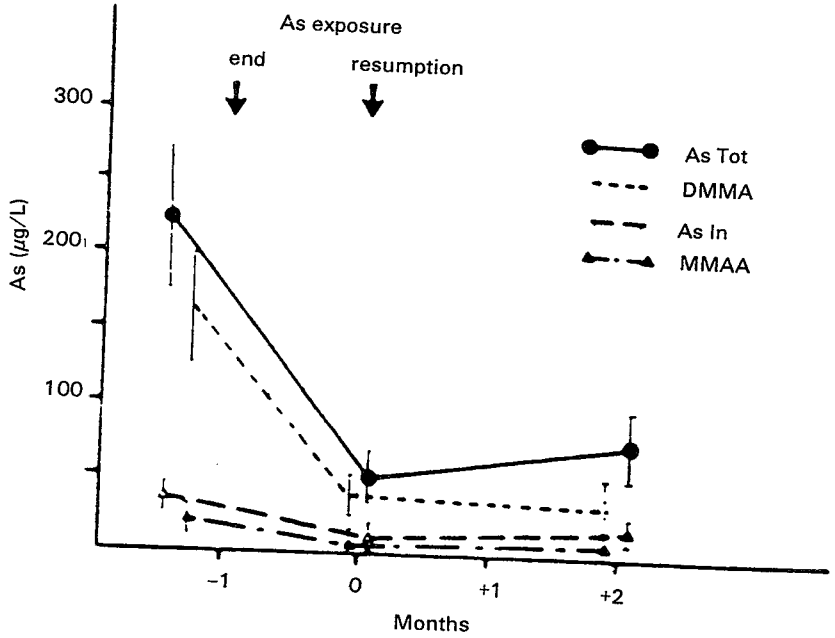
Foa et al. (1984) measured blood and urinary metabolites of arsenic in 40 glass workers exposed to high levels of arsenic and in 148 control subjects drawn from the general population. These researchers found a broad range and standard deviation for each metabolite in the blood and urine. Perhaps the most significant finding in this study was that, although many of the subjects were good methylators, each group contained subjects with clearly reduced methylation capacity as seen by the profile of metabolites. For the glass workers, both blood and urine concentrations of total arsenic were increased in proportion to the exposure, although metabolite profiles were comparable.

Foa et al. (1984) also selected a group of five glass workers with high urinary arsenic concentrations and suspended their exposure for one month. Urinary concentrations of arsenic and its methylated metabolites decreased with time nearly to that of the control population. However, when high exposure was resumed, only a moderate increase was seen for inorganic arsenic and its methylated metabolites. Two months after exposure resumed, urinary concentrations of total arsenic were still diminished relative to daily exposure (Figure E-5). Furthermore, day-to-day and morning-to-evening sampling showed only the slightest variation in concentration of inorganic arsenic, with no variation in concentration of its methylated metabolites. This appears to indicate that full methylation capacity for high exposures takes several months to build up and that any accommodation the body had made to very high arsenic levels is rapidly lost. Comparing their observations with human studies in other laboratories, these researchers postulated that the time course of excretion of metabolites indicates a saturable mechanism for the methylation of arsenic.

In a very recent study, Vahter (1986) compared urinary arsenic metabolites in smelter workers having high chronic exposures to those in a general population of non-fish eaters in Sweden. The profile of metabolites was strikingly similar (inorganic arsenic:MMA:DMA was 18%:16%:65% and 19%:20%:61%, respectively) and implied the occurrence of long-term accommodation to high levels of arsenic by the smelter workers.

In summary, similar patterns of enzymatic methylation have been demonstrated in both animals and humans. Short-term studies demonstrate that these enzymatic detoxification pathways are saturable as noted above. However, the human studies demonstrate a long-term accommodation pattern such that occupationally exposed people eliminate inorganic arsenic, MMA, and DMA in the same relative proportions as the general population or lightly exposed worker groups. Although the pattern of accommodation is consistent with traditional clinical observations of arsenic toxicology, the panel could not find any research that would enable the mechanism of accommodation to be elucidated. Finally, a number of researchers observed that methylation capacities in large populations can be highly variable.

Figure E-5. **Urinary excretion of arsenic (As) and its metabolites in glass workers with prolonged exposure to arsenic trioxide, after suspension and resumption of exposure. Values are means ±SD of five subjects.** Source: Foa et al., 1984.



IV. Pharmacokinetics of Arsenic Metabolism and Its Implications for Oncogenicity

Although most forms of arsenic to which people are commonly exposed are biologically available, inorganic arsenic is the most toxic. Inorganic arsenic is methylated enzymatically in the liver prior to its elimination in the urine. When the methylation capacity of the liver is exceeded, exposure to excess levels of inorganic arsenic can lead to increased and long-term deposition in certain target tissues, namely the liver, lung, skin, bladder, and gastrointestinal tract.

One can speculate that the methylation capacity may be exceeded at lower levels of arsenic exposure in the segments of the human population that are poor methylators due to genetic disposition or in groups consuming poor or protein- deficient diets. This may explain the anomalies noted by

Enterline in the manifestation of carcinogenic response in epidemiological studies of certain highly exposed groups (U.S. EPA, 1987).

Long-term accommodation to arsenic (on the order of several months or more) appears to take place in occupationally exposed worker populations as demonstrated by similar profiles of arsenic metabolites in the urine over a wide range of exposures. However, blood levels from high chronic exposure to arsenic (in excess of 200 µg/day) indicate that the accommodation may not be complete. However, even if the human body accommodates to chronically elevated arsenic levels, the internal tissues are nonetheless exposed to much more inorganic arsenic over long periods of time. Furthermore, the ability of the human organism to handle more than 500 or 600 µg/day may constitute a stress to the body. An improved understanding of these homeostatic mechanisms is critical to improving the cancer dose-response assessment.

Appendix C summarizes data on elevated rates of cancer of the liver, lung, and bladder in Taiwan and also notes the occurrence of internal tumors in the Fierz study. Extrapolating from the studies on protein-deficient animals, one would expect liver cancer to be especially prevalant in protein-deficient human populations. Future work may show whether the deposition patterns are matched by confirmed incidence of internal cancer.

IX. References

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