

Determination of the possible requirement and reference dose levels for arsenic in humans¹

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Studies with rats, hamsters, chicks, goats, and minipigs have indicated that arsenic is an essential element. The form of arsenic that is biologically important or performs an essential role is unknown. The determination of the reference dose, however, is based upon, or is concerned with, inorganic arsenic. Because most essentiality studies use inorganic arsenic as a supplement, the possible requirement of arsenic is also based on studies with the inorganic form. Absorption of inorganic arsenic from the gastrointestinal tract correlates well with the solubility of the compounds ingested.

In humans and most experimental animals, more than 90% of inorganic arsenate and arsenite is absorbed. Once absorbed, inorganic arsenic is transported to the liver where it is methylated in humans and most animals to monomethylarsonic acid and dimethylarsinic acid. The methylation of arsenic can be modified by changing the glutathione, methionine, or choline status of an animal. If the ingestion of arsenic is low, no tissue significantly accumulates arsenic. The excretion of ingested arsenic is rapid, urine being the major route. Urinary excretion is apparently the major homeostatic mechanism for maintaining a relatively constant body content of arsenic.

Criteria or indicators for the essentiality of an element include the following: (i) has a relatively low molecular weight and forms chelates, (ii) is ubiquitous and present in significant quantities in animals, (iii) is relatively nontoxic, and (iv) has homeostatic mechanisms of control. The item that is generally used to allow the conditional acceptance of essentiality for an element with unknown biochemical function is to show that a dietary deficiency consistently results in a reversible or preventable reduction in optimal biological function.

In 1975, Nielsen et al (1) found that arsenic deprivation elevated perinatal mortality and depressed the growth of rat pups. About the same time, Anke et al (2) found that only 58% of arsenic-deprived

goats and 62% of arsenic-deprived minipigs produced offspring compared with 92% and 100% of the respective controls. The offspring from the arsenic-deprived animals exhibited depressed growth and elevated perinatal mortality rates. Some lactating goats died, apparently from myocardial abnormalities (3).

In 1978, Nielsen & Shuler (4) found that arginine supplementation ($20 \text{ g} \cdot \text{kg diet}^{-1}$) to chicks resulted in more severe and earlier arsenic deprivation signs. Because the methyl group from methionine is important in arginine metabolism, the hypothesis was formed that arsenic has a physiological role that affects or is affected by methionine, and thus altering methionine metabolism should affect arsenic deprivation signs. This result was indeed obtained in numerous studies (5). In rats that were fed supplemental guanidoacetic acid, growth was significantly decreased in arsenic-deprived, compared with arsenic-supplemented, rats. A similar result occurred with chicks. Guanidoacetic acid utilizes a methyl group in its metabolism. Thus supplementation of this compound can alter labile methyl status. Arsenic deprivation has been shown to affect three enzymes (two are involved in methylation) necessary for phosphatidylcholine biosynthesis. In both rats and hamsters, arsenic deprivation depressed the concentration of taurine in plasma. Taurine is a product of methionine metabolism. Arsenic deprivation has been shown to decrease the polyamine content and specific activity of S-adenosylmethionine decarboxylase in rat liver. Thus there are numerous studies which indicate that arsenic is essential and that the physiological role of arsenic is influenced or associated with methionine metabolism.

Therefore, there is apparently a need for arsenic. This need becomes the most evident when an organism is exposed to a stressor that affects sulfur amino acid metabolism. Thus the safe intake of arsenic should be high enough to cover a nutritional need.

Only data from animal experiments are available for estimating the possible requirement for arsenic. Several experiments have been performed to ascertain the response of arsenic deprivation signs in chicks and rats fed graded amounts of supplemental arsenic. These experiments indicated that less than $50 \text{ ng arsenic} \cdot \text{g diet}^{-1}$ was needed to prevent deprivation signs. The minimal need seemed to be near $25 \text{ ng} \cdot \text{g}^{-1}$. These studies utilized diets containing

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20% protein, 9% fat, 60% carbohydrate, and 11% fiber, minerals and vitamins, and contained about 4000 kcal · kg⁻¹. Thus animal needs for arsenic were met by 12.5 to 25 µg · 2000 kcal⁻¹. The daily amount of food an average person consumes has an energy value of about 2000 kcal. Therefore an adequate intake of arsenic for humans may be near 12 to 25 µg daily. This value corresponds to mean dietary intakes which have been determined to range from 11.5 to 40 µg · d⁻¹. The safe upper limit of arsenic intake is higher than 40 µg · d⁻¹. The Food and Agriculture Organization of the World Health Organization determined a maximum daily load of 2 µg · kg body weight⁻¹ for arsenic. For a 70-kg person this value translates to 140 µg · d⁻¹. Furthermore, Buchet et al (6) showed that the methylation capacity of humans was reduced only when oral intakes exceeded 250 µg · d⁻¹. Thus the safe upper limit of arsenic intake could possibly be in the range of 140 to 250 µg · d⁻¹.

The determination of the reference dose (RfD) was based on the epidemiologic study in Taiwan summarized by Tseng (7). The supporting experiments used were those of Hindmarsh et al (8), Cebrián et al (9), and Southwick et al (10). All of these studies had shortcomings. In the determination of the RfD, the possible carcinogenicity of arsenic was not considered. The Taiwan data showed increased incidence of hyperpigmentation and keratosis with age and dose. The skin data formed the most sensitive end point. Thus the low dose in this study was considered to be the lowest-observed adverse-effect level (LOAEL). The reference group described for the Taiwan study showed no evidence of skin lesions. This group was utilized for determining the no-observed adverse-effect level (NOAEL). The arithmetic mean of the arsenic concentration in the wells used by the individuals in the NOAEL group was 9 µg · l⁻¹. The arithmetic mean of the arsenic concentration in the wells used by the individuals in the LOAEL group was 170 µg · l⁻¹. The actual NOAEL of 0.8 µg · kg⁻¹ · d⁻¹ was calculated by multiplying 9 µg · l⁻¹ by a water intake of 4.5 l · d⁻¹, adding 2 µg · d⁻¹ intake of arsenic through food, and dividing by an average

body weight of 55 kg. A similar calculation was used to derive the actual LOAEL of 14 µg · kg⁻¹ · d⁻¹. The uncertainty factor of 3 was used to account for both the lack of data to preclude reproductive toxicity as a critical effect and to account for some uncertainty in whether the NOAEL of the critical study (Taiwan study) accounted for all sensitive individuals. With a NOAEL of 0.8 µg · kg⁻¹ · d⁻¹, an uncertainty factor of 3, and a modifying factor of 1, the RfD comes to 0.3 µg · kg body weight⁻¹ · d⁻¹. For a 70-kg person this level calculates to 21 µg · d⁻¹. Thus the possible requirement and the RfD of arsenic are essentially the same.

References

1. Nielsen FH, Givand SH, Myron DR. Evidence of a possible requirement for arsenic by the rat. *Fed Proc* 1975;34:923.
2. Anke M, Grün M, Partschefeld M. The essentiality of arsenic for animals. In: Hemphill DD, ed. *Trace substances in environmental health*. Columbia, MO: University of Missouri, 1976:403—9.
3. Anke M. Arsenic. In: Mertz W, ed. *Trace elements in human and animal nutrition*. Orlando, FL: Academic Press, 1986:347—72.
4. Nielsen FH, Shuler TR. Arsenic deprivation studies in chicks. *Fed Proc* 1978;37:893.
5. Uthus EO. Evidence for arsenic essentiality. *Environ Geochem Health* 1992;14:55—8.
6. Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. *Int Arch Occup Environ Health* 1981;48:111—8.
7. Tseng W-P. Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic. *Environ Health Perspect* 1977;19:109—19.
8. Hindmarsh JT, McLetchie OR, Heffernan LPM, Hayne OA, Ellenberger A, McCurdy RF, et al. Electromyographic abnormalities in chronic environmental arsenicalism. *J Anal Toxicol* 1977;1:270—6.
9. Cebrián ME, Albores A, Aguilar M, Blakely E. Chronic arsenic poisoning in the North of Mexico. *Human Toxicol* 1983;2:121—33.
10. Southwick JW, Western AE, Beck MM, Whitley T, Isaacs R, Petajan J, et al. An epidemiological study of arsenic in drinking water in Millard County, Utah. In: Lederer WH, Fensterheim RJ, ed. *Arsenic: industrial, biomedical, environmental perspectives*. New York, NY: Van Nostrand Reinhold Company, 1983: 210—25.

Editors' comment

This paper discusses the possible requirement and reference dose (RfD) for humans on the basis of interesting studies which indicate that it is possible to produce arsenic deprivation in animals. However, such studies involve several methodological problems, and no biochemical function of arsenic has been identified — neither has the biologically ac-

tive form. Moreover, there is no known deficiency state in humans. Thus the editors think that it is premature to advance a human requirement for arsenic even if it is given only as a "possible requirement."

The Editors