

## Bioavailability of Inorganic Arsenic from Bog Ore-containing Soil in the Dog

Kees Groen,<sup>1</sup> Huub A.M.G. Vaessen,<sup>2</sup> Jan J.G. Kliest,<sup>3</sup> Jan L.M. de Boer,<sup>4</sup> Ton van Ooik,<sup>2</sup> Arie Timmerman,<sup>5</sup> and Ron F. Vlug<sup>5</sup>

<sup>1</sup>Unit Biotransformation, Pharmacokinetics and Toxicokinetics, <sup>2</sup>Laboratory for Residue Analysis, <sup>3</sup>Environmental Incidents, <sup>4</sup>Laboratory for Organic Analytical Chemistry, and <sup>5</sup>Central Animal Laboratory, National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands

In general, in toxicological risk assessment of environmental contaminants in humans and mammals, there is little information available on the bioavailability of toxic compounds. Applying inadequate corrections for incomplete absorption can lead to considerable overestimation of the internal dose. Recent investigations in cows on the bioavailability of dioxins bound to fly ash have clearly shown that the bioavailability of the different dioxin congeners strongly depends on the matrix to which the dioxins are bound (1). Mean bioavailability of dioxins from fly ash in cows is about 1%, which is considerably less than the 30% bioavailability of dioxin congeners from oil (2).

A situation analogous to that for the dioxins may hold true for metals. In some parts of the Netherlands, bog ore-containing soils predominate, which have natural arsenic levels that exceed, by a factor of 10, existing Dutch standards for maximum allowable levels for inorganic arsenic in soil, currently set at 50 mg/kg dry weight. In setting the tolerable intake of arsenic in humans, bioavailability from soil was assumed to be equal to that from a solution as used in toxicity studies. The main problems in setting the standards were that the limit setters took into account neither the form of arsenic nor route of exposure or the toxicological effects of its different forms (3). In a recent paper (4), adjusted, toxicologically based soil clean-up criteria have been proposed, taking into account differences in species, bioavailability, etc.

Bioavailability of inorganic arsenic from solutions was found to be more than 90% (5–9). However, as shown in Figure 1, arsenate (the most common form of arsenic in soil) is specifically bound to bog ore (10). This makes the assumption that the bioavailability of arsenic from bog ore-containing soil is equal to that from solutions questionable.

The aim of the present study was to examine whether there is a scientific basis for reconsidering the present practice of risk assessment for arsenic in soil from residential areas. For this purpose, dogs were used as an animal model because they are relatively easy to administer large amounts of soil. Furthermore, the rat is not a suitable animal model for studies on arsenic because in this species, 50% of the arsenic accumulates in the erythrocytes (11), which is not the case in dog and man.

We collected soil from an area in Doetinchem in the province of Gelderland, the Netherlands. Samples were taken at 60 and 80 cm depth. Detailed information about this area is available from earlier investigations (12). Soil samples were stored at 4°C until use.

For intravenous injection, we prepared a solution with a final concentration of 2 mg As/ml in saline using As<sub>2</sub>O<sub>5</sub> (Tritisol lot no. 09014068, Merck, Darmstadt, Germany).

The experiments were performed using three male and three female beagles (DoBe) from the cohort of the Unit Biotransformation, Pharmacokinetics and Toxicokinetics, National Institute of Public Health and Environmental Protection, the Netherlands. These animals were kept under conventional conditions. Their weight was between 10 and 15 kg and their age between 2 and 7 years. Individual data are given in Table 1. The dogs received 200 g of commercially available food twice daily. The experiment was performed according to a two-way crossover design, allowing each dog to be its own reference, and excluding the time factor as a possible cause of differences in results.

The bioavailability of arsenic from soil was related to an intravenous (IV) administered dose of arsenic. The IV dose was 2 mg As, administered as As<sub>2</sub>O<sub>5</sub> in saline via the vena cephalica. For oral administration,

In some parts of The Netherlands, bog ore-containing soils predominate, which have natural arsenic levels that exceed, by a factor of 10, existing standards for maximum allowable levels of inorganic arsenic in soil. These standards are based on the assumption that in humans the bioavailability of arsenic from ingested soil is equal to that from an aqueous solution. In view of the regulatory problem that the arsenic levels of these soils present, we questioned the validity of this assumption. To obtain a more realistic estimate, the bioavailability of inorganic arsenic from soil in a suitable animal model was studied. In this report, a study performed in six dogs in a two-way crossover design is presented. The dogs received orally, in random order, arsenic both as an intravenous solution and as arsenic-containing soil.

During a 120-hr period after administration, urine was collected in 24-hr fractions. Levels of arsenic were determined using a method of wet digestion, isolation and complexation of arsine, followed by molecule absorption spectrometry. Within 120 hr after intravenous administration, 88 ± 16% of the dose was excreted renally. After oral administration of arsenic-containing soil, only 7.0 ± 1.5% was excreted renally. From the urinary excretion data for these two routes of administration, the calculated bioavailability of inorganic arsenic from soil was 8.3 ± 2.0%. The results from this study demonstrate the need to reconsider the present risk assessment for arsenic in soil. *Key words:* arsenic, bioavailability, excretion, soil, toxicokinetics. *Environ Health Perspect* 102:182–184(1993)

soil from 80 cm depth was used because this sample was more homogeneous than the sample from 60 cm. Each animal received 20 g of soil mixed with 200 g of food. Dose levels for arsenic in soil were chosen based on data obtained in a pilot experiment. The objective was to obtain comparable plasma levels of arsenic after both routes of administration.

Before each administration, the animals were fasted for 16 hr. Administration always took place between 0900 and 1000 hr. At 1600 hr the day of administration and on every nonadministration day at 0900 am and 1600 hr, the animals received 200 g of food. During the experiment, tap water was available *ad libitum*. The dogs were individually housed in metabolism cages, and during a 120-hr period after administration, urine was

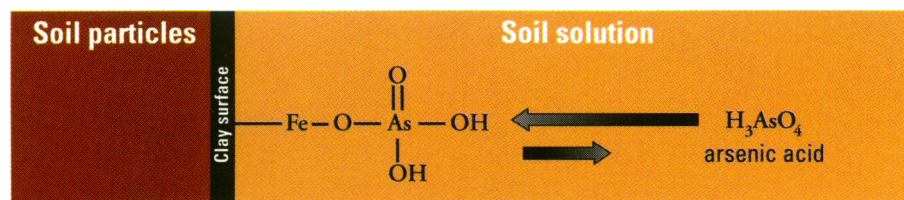


Figure 1. Schematic representation of the binding of arsenic to bog ore (10).

Address correspondence to K. Groen, Unit Biotransformation, Pharmacokinetics and Toxicokinetics (Pb 10), National Institute of Public Health and Environmental Protection, PO Box 1, 3720 BA Bilthoven, the Netherlands.

We thank Henk J.G.M. Derks for helpful discussions.

Received 28 June 1993; accepted 26 October 1993.

**Table 1.** Characteristics of the dogs used in the experiment to determine the bioavailability of arsenic from soil

Name	Gender	Session 1			Session 2		
		Weight (kg)	Route	Dose (mg As)	Weight (kg)	Route	Dose (mg As)
Xerxes	M	10.9	PO	6.74	11.9	IV	2.00
Dombo	M	14.1	IV	2.00	13.6	PO	6.67
Castor	M	13.9	IV	2.00	13.9	PO	6.64
Plekky	F	11.2	PO	7.01	11.6	IV	2.00
Anonymus	F	11.3	IV	2.00	11.8	PO	7.35
Ursula	F	11.6	PO	6.57	11.7	IV	2.00

Abbreviations: IV, intravenous; PO, oral.

caught in 24-hr fractions in glass bottles containing 5 ml of 4 M nitric acid. Urine samples were stored at -18 °C until analysis.

We determined arsenic in soil using atomic emission spectrometry with inductively coupled plasma (ICP-AES). The apparatus used was a Perkin Elmer ICP 6000 and instrumental settings were as recommended by the manufacturer. The spectral line was the 189.042 line employing background correction and nitrogen flush for the spectrometer to reduce light absorption at this wavelength.

The soil samples (1–3 kg each) were hand-homogenized using a plastic spatula, after which four subsamples of about 5 g each were taken from each sample. The samples were processed as received, i.e., in wet condition. The subsamples were digested with *aqua regia* under reflux (13). In this procedure, 4 ml of nitric acid, 65%, 12 ml of hydrochloric acid, 37%, and 50 ml of distilled water were added to the sample, and the mixture was boiled for 2.5 hr. After cooling down, we diluted the digests with water to 100 ml. After decantation, the digests were ready for analysis.

Quality control included the digestion of a standard reference material (soil-5 of the IAEA Vienna), application of standard addition to the digests to check for matrix-induced sensitivity changes in the determi-

native step, and a blank determination to check for the reagents' purity. The detection limit of the procedure was 1 µg/g. Arsenic measured in soil-5 was 97 µg/g, being well within the 95% confidence interval of 93.9 ± 7.5 µg/g. Recovery of the standard addition experiments was 102 ± 6 %, indicating that no matrix-induced sensitivity changes were present. Arsenic was not detected in the reagent blank.

We determined levels of total arsenic in urine, food, and the solution for IV injection using a procedure of wet digestion of arsenic to As(V), reduction to As(III), isolation and complexation of arsine (AsH<sub>3</sub>) with silver diethyldithiocarbamate (AgDDTC), followed by molecular absorption spectrometry (MAS). Absorption was measured at 526 nm. Sample sizes were taken depending on the expected arsenic content, resulting in amounts of arsenic within the linear range of the method (i.e. 0.5–20 µg per test solution). The validity of this method has been demonstrated for several products and biological materials, such as fruit (14). Furthermore, the arsenic content in several standard reference materials has been determined using this method (15). In the present study, the levels of arsenic in eight urine samples were determined in duplicate from separate test portions and in two samples by duplicate measurement in the same digest.

**Table 2.** Excretion of arsenic in urine (percent of dose) after intravenous and oral administration

Name	Urine fraction (hr)					Total
	0–24	24–48	48–72	72–96	96–120	
<b>Intravenous</b>						
Xerxes	79	8.5	1.5	2	1.5	92.5
Dombo	88	4	3.5	1	1	97.5
Castor	78.5	8	3	1	1	91.5
Plekky	90.5	5.5	2.5	1.5	1.5	101.5
Anonymus	41	10	3.5	1	1.5	57
Ursula	74.5	7	2	1	1.5	86
Mean	75	7.2	2.67	1.25	1.33	88
SD	18	2.2	0.82	0.42	0.26	16
<b>Oral (soil)</b>						
Xerxes	3.41	2.23	0.59	0.15	0.15	6.53
Dombo	6	1.5	0.6	0.45	0.45	9.00
Castor	3.31	1.66	0.75	0.6	0.3	6.52
Plekky	4.85	0.43	0.14	0.14	0.14	5.70
Anonymus	4.35	1.09	0.28	0.28	0.28	6.28
Ursula	3.96	2.13	2.13	0.15	0.15	8.52
Mean	4.2	1.5	0.75	0.30	0.25	7.0
SD	1.0	0.67	0.71	0.19	0.13	1.5

Total content of arsenic in food was determined in samples from the two batches used during the experiment. Results were 0.063 and 0.059 mg/kg, respectively. The arsenic content in the solution for IV injection was 2.0 mg/ml.

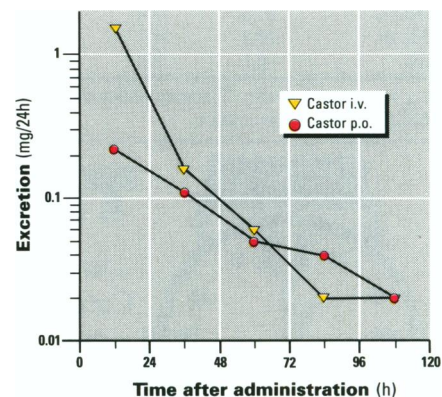
Comparison of the excretion half-lives after IV and oral administration of arsenic was performed using the Student's *t*-test on log-transformed values.

From the results of the determination of arsenic in soil, it was obvious that at 60 cm depth, arsenic was less homogeneously distributed (concentration 323 ± 74 µg/g wet weight) than at 80 cm depth (339 ± 19 µg/g wet weight). Therefore, the latter was used in the animal experiments. From previous reports, we knew that arsenate was the predominant species in soil from this area (12,13).

In Table 2, the data for the excretion of arsenic in urine after IV and oral administration are presented. After IV administration, most of the dose appeared to be excreted within 24 hr. Within 120 hr after administration, almost the entire dose was recovered in urine of five out of six dogs. Only one dog, Anonymus, had a low urinary recovery of 57% within 120 hr.

After administration of arsenic containing bog-ore mixed with food, no more than 7.0 ± 1.5% of the dose was recovered in urine within 120 hr.

Typical excretion curves after IV and oral administration are shown in Figure 2. After IV administration, two phases in the excretion curve are observed. The first part of the curve shows a half-life of 8.1 ± 3.7 hr, whereas the second part of the curve shows a half-life of 28.4 ± 8.7 hr. After oral administration of arsenic-containing soil, only an excretion phase with a half-life of 18.7 ± 5.2 hr was observed. The terminal half-lives of excretion were not significantly different between the routes of administration (Table 3).



**Figure 2.** Typical excretion curves of arsenic in urine after administration of inorganic arsenic, either as an intravenous injection (i.v.; 2.0 mg) or orally as a contaminant in soil (p.o.; 6.64 mg).

Bioavailability of arsenic from the administered soil was calculated using the equation:

$$\frac{A_{(0-120)po}}{A_{(0-120)iv}} \times \frac{D_{iv}}{D_{po}} \times 100\% = F$$

where  $A_{(0-120)po}$  and  $A_{(0-120)iv}$  are the amounts of arsenic excreted in urine within 120 hr after oral and IV administration, respectively.  $D_{po}$  and  $D_{iv}$  are the oral and IV dose, respectively.

Bioavailability of arsenic from bog ore-containing soil was  $8.3 \pm 2.0\%$ . Individual data are given in Table 3.

The study described here was performed to determine the bioavailability of arsenic from bog ore-containing soil. The dog was used as an animal model for this objective. This type of study was triggered by the discussion about whether to reconsider the present regulation for arsenic in soil from domestic areas, since in some areas, natural arsenic levels significantly exceed existing standards (4). In general, regulation is, among other things, based on the assumption that the bioavailability of contaminants from soil is the same as the bioavailability from a solution or from food. This assumption is questionable because the matrix can significantly influence the bioavailability.

Based on literature data (5,16,17), the study was designed assuming that excretion of arsenic only takes place renally. This was confirmed in our study after IV administration of arsenic. The values for the elimination half-life were comparable to those

**Table 3.** Excretion rate and bioavailability of inorganic arsenic

Name	$t_{1/2}$ (hr)		F (%)
	IV	PO	
Xerxes	33.9	15.5	7.1
Dombo	30.8	19.2	9.2
Castor	22.7	28.7	7.2
Plekky	37.8	14.3	5.6
Anonymus	13.7	17.2	11.0
Ursula	31.3	17.0	9.9
Mean	28.4	18.7	8.3
SD	8.7	5.2	2.0

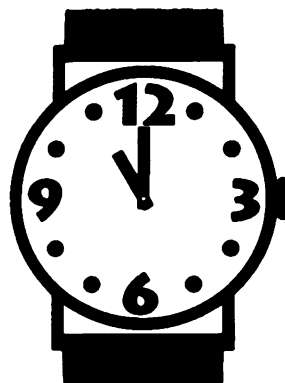
$t_{1/2}$ , excretion half-life; F, bioavailability of arsenic based on urinary excretion.

reported in literature (11). Our data show that only about 8% of arsenic from bog ore-containing soil is absorbed from the gastrointestinal tract and excreted into urine. Thus, bioavailability of arsenic from soil is much lower than from a solution, either containing As(III) or As(V) (5,8,9). Seventy-three percent of orally administered  $As_2O_3$  in water was recovered in urine of female monkeys (5). After ingestion of dissolved inorganic As(V) by two volunteers, 64–69% of the dose was excreted into urine within 166 hr (9). However, several factors, such as species tested and dietary status, could modify the extent of absorption.

Comparing data from the present study with those from a study using a gastric juice simulation test reveals that the simulation test underestimated the release of arsenic from bog ore-containing soil. In the gastric simulation test, a release of less than 1% was found (12). The results obtained from the present investigation on the bioavailability of inorganic arsenic from bog ore-containing soil in dogs stress the need to reconsider the present practice of risk assessment for arsenic in soil in residential areas.

#### REFERENCES

- Olling M, Berende PLM, Derks HJGM, Everts H, de Jong APJM, Liem AKD. Bioavailability of dioxins and furans from fly ash in the cow. RIVM report 328904.004. Bilthoven, the Netherlands: National Institute of Public Health and Environmental Protection, 1992.
- Olling M, Derks HJGM, Berende PLM, Liem AKD, de Jong APJM. Toxicokinetics of eight  $^{14}C$ -labelled polychlorinated dibenzo-*p*-dioxins and furans in lactating cows. *Chemosphere* 23:1377-1385 (1991).
- Moen JET, Cornet JP, Evers CWA. Soil protection and remedial actions: criteria for decision making and standardisation of requirements. In: Contaminated soil: Proceedings of the International TNO Conference on Contaminated Soils (Assink JW, Van den Brink WJ, eds). Dordrecht, the Netherlands: Martinus Nijhoff Publishers, 1986;441-448.
- van den Berg R, Denneman CAJ, Roels JM. Risk assessment of contaminated soil: proposals for adjusted, toxicologically based Dutch soil clean-up criteria. In: Contaminated soil '93 (Arendt F, Annokée GJ, Bosman R, van den Brink WJ, eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, 1993;349-364.
- Charbonneau SM, Spencer K, Bryce F, Sandi E. Arsenic excretion by monkeys dosed with arsenic-containing fish or with inorganic arsenic. *Bull Environ Contam Toxicol* 20: 470-477(1978).
- Pomroy C, Charbonneau SM, McCullough RS, Tam GKH. Human retention studies with  $^{74}As$ . *Toxicol Appl Pharmacol* 53:550-556(1980).
- Marafante E, Rade J, Sabbioni E, Bertolero F, Foa V. Intracellular interaction and metabolic fate of arsenite in the rabbit. *Clin Toxicol* 18:1335-1341(1981).
- Ishinishi N, Tsuchiya K, Vahter M, Fowler BA. Arsenic. In: Handbook on the toxicology of metals, vol 1 (Friberg L, Nordberg GF, Vou VB, eds). Amsterdam:Elsevier, 1986; 43-83.
- Johnson LR, Farmer JG. Use of human metabolic studies and urinary arsenic speciation in assessing arsenic exposure. *Bull Environ Contam Toxicol* 46:53-61(1991).
- Woolson EA. Fate of arsenicals in different environmental substrates. *Environ Health Perspect* 19:73-81(1977).
- International Programme on Chemical Safety. Environmental health criteria 18: arsenic. Geneva:World Health Organization, 1981.
- Zuurdeeg BW, van Enk RJ, Vriend SP. Herkomst van de verhoogde arseengehalten in de bodem van De Huet, Doetinchem. Utrecht, the Netherlands:Geochem-research, 1990.
- Anonymous. De belasting van de mens met arseen op ijzerrijke gronden in Gelderland. Deel I, literatuur-studie. Heerenveen, the Netherlands:Oranjewoud,1987.
- Vaessen HAMG, van Ooik A, van de Kamp CG. Some elements in domestic and imported fresh fruits marketed in the Netherlands. *Z Lebensm Unters Forsch* 193:351-355(1991).
- Vaessen HAMG, van Ooik A, Zuydendorp J. Method 15—determination of arsenic in foods and human tissues by spectrophotometry. In: Environmental carcinogens: selected methods of analysis, vol 8. Lyon:International Agency for Research on Cancer, 1986;365-375.
- Hunter FT, Kip AF, Irvine JW. Radioactive tracer studies on arsenic injected as potassium arsenite. I. Excretion and localization in tissues. *J Pharmacol Exp Ther* 76:207-220(1942).
- Hesse JM, Janus JA, Krajnc EI, Kroese ED. Integrated criteria document arsenicum effects. Appendix to RIVM-report no. 758701002. Bilthoven, the Netherlands: National Institute of Public Health and Environmental Protection, 1990.



## THE TIME HAS COME...

...to send for the latest copy of the free Consumer Information Catalog. It lists more than 200 free or low-cost government publications on topics like money, food, jobs, children, cars, health, and federal benefits. Send your name and address to:

**Consumer Information Center  
Department TH  
Pueblo, Colorado 81009**

