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Percutaneous Penetration of Inorganic Mercury from Soil: An *In Vitro* Study

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Soil has been recognized as a potential source of exposure to chemical contaminants (Kadry et al. 1995; Wester et al. 1990).

Skin is such an important route of absorption of mercury compounds that ACGIH has assigned them a skin notation (ACGIH 2000). It therefore seems advisable to establish maximum daily exposure levels for mercury in soil. In the past, areas adjacent to certain industries and smelters have been heavily contaminated by mercury.

Using an *in vitro* diffusion cell system and human skin, we studied percutaneous penetration of mercuric chloride ($HgCl_2$) at different concentrations, with particular enphasis on skin absorption from soil.

MATERIALS AND METHODS

The test apparatus consisted of a flow-through diffusion cell system (LG-1084-SPC, LGA Berkley CA) kept at a constant temperature of 37°C so that the skin surface temperature was 32°C. The exposure area was 0.95 cm^2. Flow rate through the diffusion cells was 1.2 ml/h. Samples of receiving fluid were drawn using a fraction collector (Crown Glass Co, NJ) at 1st, 2nd, 8th hour and after every 8 hours in 72 h.

Dermotomed human cadaver skin from the abdomen of white men without skin diseases was used as the membrane. Skin thickness was measured by using a micrometer (PK-1012 Mitutoyo Corporation, Japan). Samples >600 and <300 μm thick were rejected. Ten skin samples from 4 different donors were frozen at –80° and stored for a maximum of 3 months. The receiving liquid was a saline solution with 6% PEG-20 oleyl-ether and 10 mg/100 ml of gentamycin sulphate.

$^{203}HgCl_2$ with a specific activity of 1.92 mCi/mg Hg was purchased from Amersham (Buckinghamshire UK). A buffered solution prepared by adding gr 27.2 of KH_2PO_4 in ml 500 of water in order to raise the pH (pH=4) avoiding a skin damage and an air-dried loam soil were used as vehicles. In the case of the

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liquid vehicle, 20 µl of solution were applied on the skin. The soil was prepared following a technique previously described by Yang et al. (1989) using 6 gr of sand (60%), 3 gr of silt (30%) and 1 gr of clay (10%). The soil preparation involved adding 20 µl of $^{203}HgCl_2$ in the buffered solution followed by gentle manual stirring. The amount of soil was mg 40 for each cell.

Two different concentrations were used in both vehicles: 0.0088 and 0.0607 nmol/cm^3 for the buffered solution and 0.0069 and 0.1190 nmol/cm^3 for the soil, respectively. Each experiment was carried out using skin samples from the same donor. At the end of the experiment skin was washed with water and rubbed with cotton swabs. Skin samples were analysed after solubilisation in Soluene 350 (Packard) and a digestion time of 24 hours at 45-50 C°.
Skin in the assembled diffusion cells was screened for barrier integrity using the percutaneous penetration of tritiated water. As previously described (Bronaugh et al. 1986) tritiated water was applied to the surface for 20 minutes; the absorption was expressed as a percentage of the applied dose, the normal value being considered <0.29%.

Radioactivity in the skin and in the receiving phase was detected after the addition of the scintillation cocktail in a Packard scintillation counter.
The results were expressed as a percentage of absorption, flux at steady state, Kp and concentration (and percentage of the applied dose) of $^{203}HgCl_2$ in the skin at the end of the experiment.

Concerning the experiment where the liquid vehicle was used, the observed data are longitudinal data relative to two groups of cells with different applied concentrations (of size 5 and 4 cells, respectively). In order to perform analysis, we considered the increments of the percent dose at the different hours rather than the data based on cumulative percentages. It is worth noting that the analysis is twofold, *i.e.* we aimed to assess the global homogeneity of the increment overall paths in the two groups as well as the homogeneity of the increments in the two groups at each sampling time. A suitable technique for detecting such an involved structure of hypotheses may be based on a recently-proposed permutation method (Pesarin 2001, Barabesi and Fattorini 1997). By adapting this procedure to the present analysis, the overall homogeneity hypothesis of two groups in respect to the path for all sampling times is decomposed in the intersection of marginal hypothesis, each relative to the homogeneity of the two groups in respect to each single sampling time. In a first stage, the significance relative to each marginal test statistic is obtained by a permutational procedure and these values are combined in an overall test statistic. In turn, the significance of the overall test statistic is computed by means of the same permutations performed on the data. In this way the dependence structure of the marginal statistics is non parametrically captured by the permutation procedure. Hence, if the overall homogeneity hypothesis is rejected, one can assess the marginal hypothesis on which the rejection depends. It should be noted, that the method allows for the joint analysis of the variables as well as the single marginal analyses, without assuming any model for the population under study.

Table 1. Percutaneous penetration of $^{203}HgCl_2$ in a buffered solution (applied dose 0.0088 nmol/cm^3).

	Kp (cm/h)	**Steady state flux** (nmol/cm^2/h)
Cell 1	16.47×10^{-3}	0.14×10^{-3}
Cell 2	15.79×10^{-3}	0.14×10^{-3}
Cell 3	15.90×10^{-3}	0.14×10^{-3}
Cell 4	11.12×10^{-3}	0.09×10^{-3}
Cell 5	12.84×10^{-3}	0.11×10^{-3}
Mean ± DS	$14.42 \pm 2.32 \times 10^{-3}$	$0.12 \pm 0.02 \times 10^{-3}$
GM [GSD]*	$14.26\ [1.18] \times 10^{-3}$	$0.12\ [1.22] \times 10^{-3}$
Median	15.8×10^{-3}	0.14×10^{-3}

(*) geometric mean (GM) and relative standard deviation (GSD)

Table 2. Percutaneous penetration of $^{203}HgCl_2$ in a buffered solution (applied dose 0.0607 nmol/cm^3).

	Kp (cm/h)	**Steady state flux** (nmol/cm^2/h)
Cell 1	2.86×10^{-3}	0.17×10^{-3}
Cell 2	2.62×10^{-3}	0.16×10^{-3}
Cell 3	3.18×10^{-3}	0.19×10^{-3}
Cell 4	3.52×10^{-3}	0.21×10^{-3}
Mean ± DS	$3.04 \pm 0.39 \times 10^{-3}$	$0.18 \pm 0.02 \times 10^{-3}$
GM [GSD]*	$3.03\ [1.14] \times 10^{-3}$	$0.18\ [1.13] \times 10^{-3}$
Median	3.02×10^{-3}	0.18×10^{-3}

(*) geometric mean (GM) and relative standard deviation (GSD)

RESULTS AND DISCUSSION

In the receiving fluid of cells where soil was applied as vehicle, concentration of mercury was always below the detection limit. The mean mercury concentrations in the skin samples were 10.53% (SD 4.38, range 6.91-14.57%) and 15.04% (SD 10.80, range 7.40-22.68%) of the applied dose respectively when 0.0069 and0.1190 nmol/cm^3 were applied. Table 1 and 2 summarize percutaneous

Table 3. Cumulative percentages of absorption at the different hours and percentages retained in the skin samples of $^{203}HgCl_2$ in a buffered solution (applied dose 0.0088 nmol/cm^3).

	Mean ± SD	GM [GSD]*	Median	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
% 1h	0.06 ± 0.01	0.05 [1.40]	0.06	0.07	0.06	0.06	0.03	0.06
% 2h	0.12 ± 0.04	0.12 [1.38]	0.12	0.18	0.13	0.15	0.07	0.11
% 8h	0.56 ± 0.11	0.55 [1.22]	0.57	0.57	0.64	0.70	0.45	0.45
% 16h	1.14 ± 0.18	1.12 [1.19]	1.22	1.22	1.25	1.30	0.84	1.09
% 24h	1.64 ± 0.23	1.63 [1.16]	1.77	1.77	1.77	1.87	1.32	1.49
% 32h	2.17 ± 0.40	2.14 [1.21]	2.36	2.37	2.42	2.56	1.63	1.88
% 40h	2.67 ± 0.52	2.62 [1.23]	2.91	3.12	2.92	3.02	1.89	2.40
% 48h	3.14 ± 0.62	3.09 [1.24]	3.40	3.77	3.40	3.50	2.21	2.83
% 56h	3.66 ± 0.80	3.59 [1.26]	3.80	4.39	3.80	4.40	2.51	3.22
% 64h	4.18 ± 0.88	4.10 [1.25]	4.24	5.09	4.24	4.97	3.01	3.59
% 72h	4.80 ± 0.99	4.71 [1.24]	4.90	5.70	4.90	5.79	3.53	4.07
% in the skin	18.93 ± 15.15	13.02 [3.03]	12.54	11.87	40.94	27.04	2.27	12.54

(*) geometric mean (GM) and relative standard deviation (GSD)

Table 4. Cumulative percentages of absorption at the different hours and percentages retained in the skin samples of $^{203}HgCl_2$ in a buffered solution (applied dose 0.0607 nmol/cm^3).

	Mean ± SD	GM [GSD]*	Median	Cell 1	Cell 2	Cell 3	Cell 4
% 1h	0.01 ± 0.003	0.01 [1.26]	0.009	0.008	0.009	0.008	0.014
% 2h	0.03 ± 0.008	0.02 [1.39]	0.03	0.02	0.02	0.03	0.04
% 8h	0.11 ± 0.03	0.11 [1.42]	0.11	0.11	0.07	0.12	0.15
% 16h	0.20 ± 0.09	0.17 [1.94]	0.23	0.20	0.07	0.25	0.27
% 24h	0.34 ± 0.05	0.34 [1.16]	0.33	0.32	0.30	0.33	0.42
% 32h	0.48 ± 0.06	0.47 [1.13]	0.46	0.46	0.42	0.45	0.57
% 40h	0.59 ± 0.05	0.59 [1.09]	0.58	0.57	0.54	0.60	0.67
% 48h	0.69 ± 0.07	0.68 [1.10]	0.69	0.66	0.61	0.71	0.77
% 56h	0.77 ± 0.08	0.77 [1.11]	0.77	0.74	0.67	0.81	0.86
% 64h	0.85 ± 0.10	0.84 [1.13]	0.86	0.81	0.71	0.91	0.94
% 72h	0.93 ± 0.12	0.92 [1.14]	0.95	0.90	0.77	1.00	1.04
% in the skin	44.97 ± 7.88	44.44 [1.19]	44.83	43.16	35.60	46.50	54.61

(*) geometric mean (GM) and relative standard deviation (GSD)

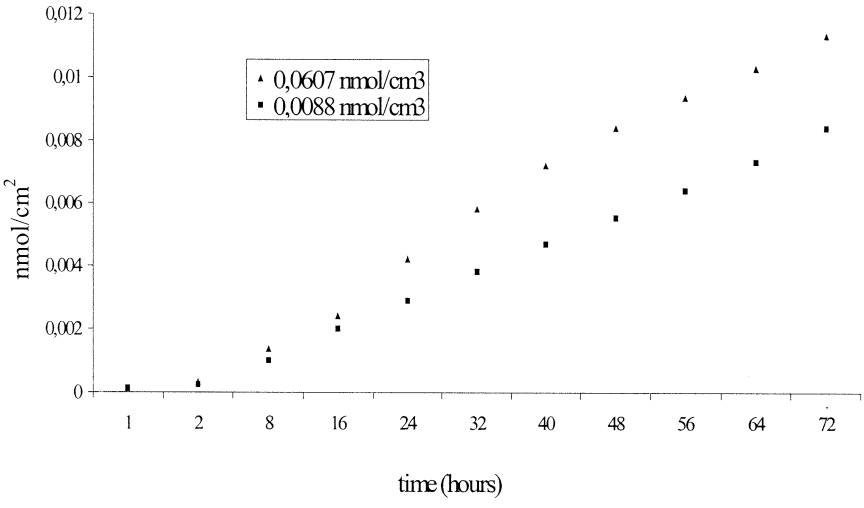

Figure 1. Cumulative percutaneous penetration as a function of time of $^{203}HgCl_2$ applied in two different concentrations in a buffered solution.

penetration data of mercury when applied in a buffered solution in 2 different concentrations. In table 3 and 4 cumulative percentages of absorption at the different hours in the different diffusion cells and percentages retained in the skin samples are reported. Increments of percentage of absorption at the different hours were significantly lower in the cells where the higher concentration of $^{203}HgCl_2$ was applied. The analysis performed on the present data shows that overall homogeneity hypothesis should be stronglyrejected since a very small p-value was obtained ($p<0.001$). Moreover, even all the marginal hypotheses relative to each single sampling time should be rejected since they display small p-values (in each case $p<0.01$). Hence, the statistical analysis leads to a strong rejection of the homogeneity hypothesis for the two groups.

Kp values were significantly lower in the cells where the higher concentration of $^{203}HgCl_2$ was applied (Mann-Whitney U test, $p<0.05$), while the percentage of the applied dose in the skin samples was significantly higher in the cells where the more concentrated solution was applied than in those with the more diluted solution (Mann-Whitney U test, $p<0.05$).
Figure 1 shows the cumulative percutaneous penetration of mercury applied in 2 different concentrations in the liquid vehicle, while in figure 2, percentages of absorption at the different hours for the 2 concentrations are reported. Figure 3 shows the increments of the percent dose at the different hours for the 2 concentrations.

Percutaneous penetration of mercury from the buffered solution seems relevant.

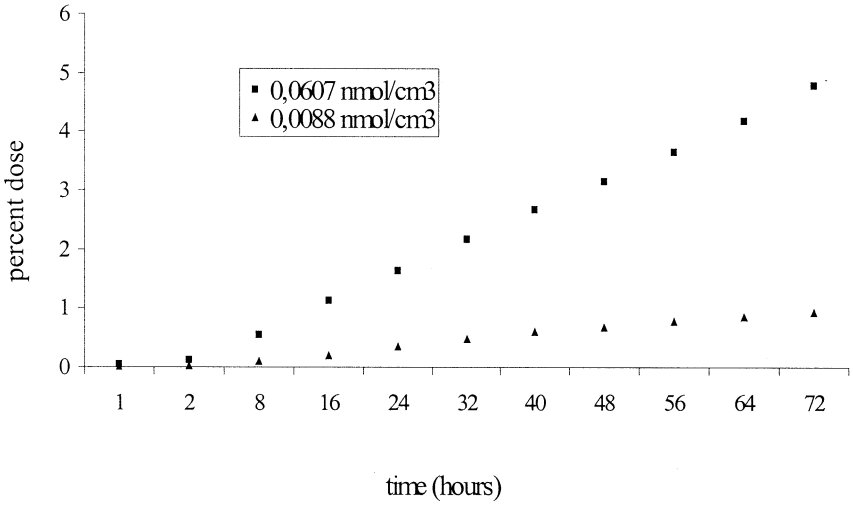

Figure 2. Cumulative percent doses as a function of time of $^{203}HgCl_2$ applied in two different concentrations in a buffered solution.

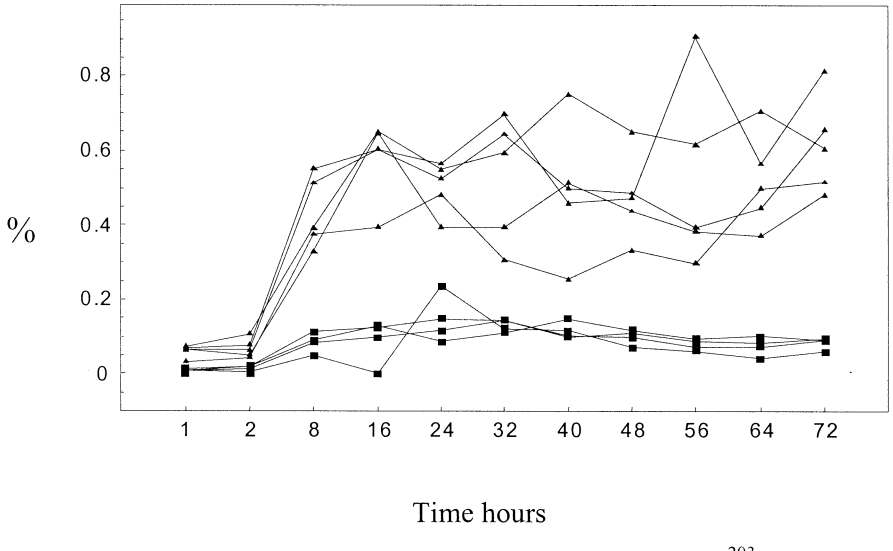

Figure 3. Increments of the percent dose at different hours of $^{203}HgCl_2$ applied in a buffered solution in two concentrations (▲ 0.0088 nmol/cm^3; ■ 0.0607 nmol/cm^3).

Obviously cumulative percutaneous penetration is higher in the cells where the higher concentration of $^{203}HgCl_2$ was applied. However increments of percent dose are significantly lower in the cells where the higher concentration was deposited. The slower percutaneous penetration from the higher concentration solution is confirmed by the Kp values in the 2 different conditions. This is in contrast with the common belief that the concentration of chemicals cannot influence Kp values since Kp is a constant derived from the first law of Fick.
The Fick's law applies to percutaneous penetration if this can be considered as an uniform diffusion process through the stratum corneum. Thus Kp of mercury seems to be affected by increasing the applied dose, probably because the solubility into the stratum corneum is a rate-limiting process. Formation of stratum corneum reservoir, and loss of sink conditions can also play a role in this. Formation of reservoir is confirmed by the concentration of $^{203}HgCl_2$ measured in the skin at the end of the experiment. In the cells where the more concentrated solution was applied, the percentage of the applied dose in the skin samples was higher than in those with the more diluted solution. This can be explained by the high affinity of mercury for the skin and should be taken into consideration in the percutaneous penetration studies of other metals. In future research, retention in the stratum corneum could be helpfully separated from the retention in the other parts of the skin by using tape-stripping at the end of the experiment and counting that separately for radioactivity. Thus any measured radioactivity in the remaining skin could be attributed to skin absorption.

In literature there is very little in vitro data on percutaneous penetration of mercury through human skin. In the 1960s Wahlberg and his associates, in a series of at least nine reports, measured the absorption of mercury through guinea pig skin and, in one case, also through human skin (Guy et al. 1999). They found that Kp of $HgCl_2$ on guinea pig *in vivo* was smaller at the lowest and highest applied concentrations, but without explanation for this observation. In *in vitro* studies with both guinea pig skin and human skin, in the end of the experiment the absorption rate was less than 40% of the initial rate. This seems to be related to a nonlinear absorption and can explain changes of Kp. With buffered solution we observed steady-state conditions and Kp did not change during the experiment. Kp values cannot be compared with those obtained by Wahlberg (1965) because of the different experimental conditions (e.g. they did not use dermotomed skin and measured the decrease of radioactivity from the site of application).

Various studies demonstrated that chemicals are poorly absorbed through the skin from solid vehicles (Yang et al. 1989; Wester et al. 1993; Sartorelli et al. 2001). Absorption of inorganic mercury by skin from contaminated soil was measured, but penetration through the section of skin could not be demonstrated in this study. This should be confirmed in *in vivo* studies on human volunteers that are not easily realizable. However comparison with results obtained using a liquid vehicle and the same experimental conditions shows a slower percutaneous penetration from soil.

Some of the mercury absorbed by the skin would likely be distributed systemically over a period of time. Skin contamination with soil containing inorganic mercury may be a dermal risk. Distribution throughout the body could be evaluated using in vivo models.

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