In Vivo Percutaneous Absorption of Boric Acid, Borax, and Disodium Octaborate Tetrahydrate in Humans Compared to in Vitro Absorption in Human Skin from Infinite and Finite Doses

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Literature from the first half of this century report concern for toxicity from topical use of boric acid, but assessment of percutaneous absorption has been impaired by lack of analytical sensitivity. Analytical methods in this study included inductively coupled plasma-mass spectrometry which now allows quantitation of percutaneous absorption of $^{10}$B in $^{10}$B-enriched boric acid, borax, and disodium octaborate tetrahydrate (DOT) in biological matrices. This made it possible, in the presence of comparatively large natural dietary boron intakes for the in vivo segment of this study, to quantify the boron passing through skin. Human volunteers were dosed with $^{10}$B-enriched boric acid, 5.0%, borax, 5.0%, or disodium octaborate tetrahydrate, 10%, in aqueous solutions. Urinalysis, for boron and changes in boron isotope ratios, was used to measure absorption. Boric acid in vivo percutaneous absorption was 0.226 (SD = 0.125) mean percentage dose, with flux and permeability constant ($K_p$) calculated at 0.009 μg/cm²/h and $1.9 \times 10^{-7}$ cm/h, respectively. Borax absorption was 0.210 (SD = 0.194) mean percentage of dose, with flux and $K_p$ calculated at 0.009 μg/cm²/h and $1.8 \times 10^{-7}$ cm/h, respectively. DOT absorption was 0.122 (SD = 0.108) mean percentage, with flux and $K_p$ calculated at 0.01 μg/cm²/h and $1.0 \times 10^{-7}$ cm/h, respectively. Pretreatment with the potential skin irritant 2% sodium lauryl sulfate had no effect on boron skin absorption. In vitro human skin percentage of doses of boric acid absorbed were 1.2 for a 0.05% solution, 0.28 for a 0.5% solution, and 0.70 for a 5.0% solution. These absorption amounts translated into flux values of, respectively, 0.25, 0.58, and 14.58 μg/cm²/h and permeability constants ($K_p$) of $5.0 \times 10^{-4}$, $1.2 \times 10^{-4}$, and $2.9 \times 10^{-4}$ cm/h for the 0.05, 0.5, and 5.0% solutions. The above in vitro doses were at infinite, 1000 μl/cm² solution. At 2 μl/cm² (the in vivo dosing volume), flux decreased some 200-fold to 0.07 μg/cm²/h and $K_p$ of $1.4 \times 10^{-6}$ cm/h, while percentage of dose absorbed was 1.75%. Borax dosed at 5.0%/1000 μl/cm² had 0.41% dose absorbed, flux at 8.5 μg/cm²/h, and $K_p$ was $1.7 \times 10^{-4}$ cm/h. Disodium octaborate tetrahydrate (DOT) dosed at 10%/1000 μl/cm² was 0.19% dose absorbed, flux at 7.9 μg/cm²/h, and $K_p$ was $0.8 \times 10^{-4}$ cm/h. These in vitro results from infinite doses (1000 μl/cm²) were 1000-fold greater than those obtained in the companion in vivo study. The results from the finite (2 μl/cm²) dosing were closer (10-fold difference) to the in vivo results. General application of infinite dose percutaneous absorption values for risk assessment is questioned by these results. These in vivo results show that percutaneous absorption of boron, as boric acid, borax, and disodium octaborate tetrahydrate, through intact human skin, is low and is significantly less than the average daily dietary intake. This very low boron skin absorption makes it apparent that, for the borates tested, the use of gloves to prevent systemic uptake is unnecessary. These findings do not apply to abraded or otherwise damaged skin. © 1998 Society of Toxicology.

There is conflicting information about the absorption of boron as boric acid and sodium borates through skin. On the one hand, Beys et al (1983) reported that boric acid is poorly absorbed through the skin, and on the other, Lewis (1990) reported that boron compounds such as boric acid are absorbed after skin contact and recommended the use of gloves and other protective garments. Questions about the penetration of borax and boron acid through skin have come from exposure to borates in houses treated for insects, from worker exposure, and consumer use of borate products. The issue of skin absorption has been raised in swimming pool risk assessment efforts, i.e., assumptions made on the amount of boric acid absorbed through the skin during swimming in a treated pool. This has prompted concern that topical use of boric acid could lead to systemic effects.

Studies of boric acid skin absorption began in the 1920s with...
the work of Kahlenberg (1924). The accuracy of these studies, as well as the subsequent ones by Pfeifer et al. (1945), John-
stone et al. (1955), Freimuth and Fisher (1958), Draize and Kelley (1959), and Fris-Hansen et al. (1982), is in doubt since they were unable to identify the presence of boron in control urine samples. The amount of boron excreted by the kidney is considered a good measure of the amount of boron absorbed systemically; approximately 98.7 urinary excretion was found by Jansen et al. (1984) over a 120-h period following intravenous administration of doses of 570 to 620 mg boric acid to healthy adult human volunteers. Boric acid is not metabolized by humans or animals and is excreted unchanged (Murray, 1995; Moore, 1997). Currently recognized mean values for boron in urine for the general population range from 0.07 to 1.80 \( \mu \)g of boron/cc of urine (Imbus et al., 1963; Abou-Shakra et al., 1989; Minoia et al., 1990). The methods of Vignnec and Ellis (1954) and Stuttgen et al. (1982) were sensitive enough to measure boron in control urine samples as well as samples from test subjects. The Vignnec and Ellis (1954) study showed that boric acid in the presence of talc was not absorbed through intact or irritated infant skin, while the Stuttgen et al. (1982) study showed that boric acid in a water emulsifying ointment was not absorbed by either normal or diseased skin. These two studies tested boric acid in combination with other substances intended to reduce its absorption. No other studies were found that had both adequate sensitivity and tested pure borate solutions.

The measurement of absorbed borate is made difficult by the presence of boron in normal diet. Boron, being an essential element for plant growth, is present in the American diet in milligram amounts (Anderson et al., 1994). It is rapidly and almost quantitatively excreted by the kidneys with a half-time of 21 h (Jansen et al., 1984). Difficulties in its measurement in biological matrices are made worse by potential contamination from the environment or the analytical procedure as well as variability in the analytical method at the low levels (a few ppb to low ppm) that must be measured (Smith et al., 1991). In the absence of convenient isotope labeling, measurement of skin absorption had to await the development of analytical methods that could overcome the difficulties faced by earlier investigators. In recent years such methods have become available. Through the use of the stable isotopes of boron, this study was able to differentiate the amount of borate absorbed from skin application from the borate consumed through diet.

Review of these past studies leads to the conclusion that there are no reliable scientific studies quantifying the skin absorbability of boric acid or inorganic borates. This study determined the in vivo percutaneous absorption of boric acid, borax, and disodium octaborate tetrahydrate (DOT) in human volunteers with normal and with sodium lauryl sulfate (SLS)-treated skin. A corollary in vitro study with human skin was done to determine if an in vitro diffusion system was predictable of in vivo absorption. Analyses were done by ICP/MS, the most sensitive method available. Boric acid, borax, and DOT were made as >99% enriched \(^{10}\)B products (\(^{10}\)B is a stable, nonradioactive isotope of boron). Boron from the environment exists as a mixture of \(^{10}\)B and \(^{11}\)B stable isotopes (approximately 19% \(^{10}\)B and 81% \(^{11}\)B).

MATERIALS AND METHODS

Test articles, \(^{10}\)B-enriched boric acid, \(^{10}\)B-enriched borax, and disodium octaborate tetrahydrate were provided by U.S. Borax Inc. (Valencia, CA). Sodium lauryl sulfate was obtained from Sigma Chemical Co. (St. Louis, MO). The dosing formulations, 5% boric acid solution, and 5% borax solution were prepared by adding the appropriate volume of water to the appropriate weighed amount of test compound. Samples were sonicated for a short time to ensure homogeneity. Ten percent disodium octaborate tetrahydrate solution was formulated by U.S. Borax Inc. Stability of the formulations (U.S. Borax Inc. and West Coast Analytical, Inc.) was shown for the course of the study.

The in vivo dose was 1.8-ml solutions over 900 cm\(^2\) skin area, or 2 \( \mu \)l/cm\(^2\), the maximum dose which would not run off the skin. The in vitro dose was either an infinite dose of 1000 \( \mu \)l/cm\(^2\) or the finite dose of 2 \( \mu \)l/cm\(^2\) to match the in vivo dose.

In vivo study. Human volunteers were recruited from the University of California, San Francisco (UCSF), and the surrounding San Francisco Bay Area community. Twenty-four normal, healthy, males or females, aged from 22 to 50 years were selected for this study. Before the study, all volunteers were instructed on how to keep a daily food diary and on what food items high in boron to avoid during the course of the study.

The studies were divided into three groups and each group contained eight volunteers. Background urine was collected for the first 4 days. Groups I, II, and III received two separate topical applications of \(^{10}\)B-enriched 5% boric acid, 5% borax, and 10% DOT solutions on their back skin, respectively. One dose was applied on day 5 under normal skin conditions and the other dose was applied on day 12 under potentially irritated skin conditions created by a 24-h application of 2% SLS solution. It was assumed that 2% SLS would cause a visible skin irritation and increase transepidermal water loss (as a measurement of skin barrier compromise). Twenty-four hours after each topical dose, residual chemical on the dosed skin site was removed by skin wash. Twenty-four hour complete urinary samples were collected daily for 17 days. In the in vivo study, it was assumed that daily urinary boron excretion provided the best estimate of boron absorption. Jansen et al. (1984) recovered a mean of >98% of \(^{10}\)B administered doses of boron in urine, while Hunt et al. (1997), in a human metabolic study, found 89-102% of ingested boron excreted in urine and no indication of boron accumulation over time. If, as indicated by some animal studies (Ku et al., 1991), boron is accumulated in bone, it must in humans be a very small percentage of the daily boron intake. Urine samples from day 1 to day 4 were used to establish base boron levels and isotope ratios in the urine. The samples from day 5 to day 11 and day 12 to the end were used to compare absorbed level under normal skin and irritated skin conditions. To evaluate the dosing site skin condition, transepidermal water loss (TEWL) measurement and skin visual scoring were taken each time before dosing (including SLS treatment) and washing. Five sites of the marked area, the upper left corner, the upper right corner, the center, the lower left corner, and the lower right corner, were used for TEWL measurement.

On day 5, the physical condition of the back skin of each volunteer was examined and then a 900-cm\(^2\) area, 30 \( \times \) 30 cm, was marked on the back. The marked area received a single topical application of 5% boric acid (group I), 5% borax (group II), or 10% DOT (group III). Each dose formulation was delivered with a 3.0-ml Eppendorf polypropylene syringe (Brinkmann Instruments, Ind., Westbury, NY). The delivered dose was quantified by weighing the syringe before and after dosing.

After topical application, the dosed area was allowed to air dry and then the volunteer was dressed in a commercial white T-shirt for 24 h. The volunteers were requested not to touch or wash the dosed area for 24 h. Thus the dosing site was protected for the 24-h dosing period. Twenty-four hours after dosing
(day 6), the T-shirt was carefully removed and the dosed site was washed using gauze pads (Sherwood Medical, St. Louis, MO), Ivory liquid soap (Procter & Gamble, Cincinnati, OH), and water. The washing procedure was as follows: (1) 50% Ivory soap solution (v/v), (2) distilled deionized water, (3) 50% Ivory soap solution (v/v), and (4–10) distilled deionized water. T-shirts and skin washes were analyzed for boron content.

On day 11, the volunteer arrived the same time of day as the first time of dosing and was treated with 1.8 ml of 2% SLS solution on the same 900-cm² area of the back. On day 12, 24 h after SLS treatment, a single topical application of 5% boric acid (group I), 5% borax (group II), or DOT (group III) was applied as on day 5. After topical application, the dosed area was allowed to air dry and then the volunteer dressed in a commercial white T-shirt for 24 h. Again volunteers were requested not to touch or wash the dosed area for 24 h.

Twenty-four hours after second dosing (day 13), the T-shirt was carefully removed and the dosed site was washed using gauze pads and liquid soap and water (50/50). The washing procedure was the same as before. T-shirts and skin washes were analyzed for boron content.

Transsepidermal water loss of the treated skin was measured by the Tewameter TM210 manufactured by Courage & Khazaka Electronic GmbH (Cologne, Germany). Five sites on the treated skin were used for each measurement; those were the upper left corner, the upper right corner, the center, the lower left corner, and lower right corner. Each measurement was taken for at least 3 min. The average value of the last 20 s and the standard deviations were recorded when the measurement value was stable. Prior to the measurement, the subject rested in the test room at least 30 min; room temperature and humidity were recorded. During the study (on day 5, day 6, day 11, day 12, and day 13) the volunteers’ skin was checked by a trained dermatologist for erythema and edema (redness and inflammation). Skin condition was scored on a graded scale of 0–5, where 0 is no observable response and 5 is blistering.

In order to avoid excess dietary boron intake, the volunteers were asked to follow some dietary restrictions during the 17-day study. Foods and beverages to avoid were dried fruits (prunes, raisins, dates, etc.) including foods containing these items: cherries, avocados, nuts (peanuts, pecans, almonds, etc.), peanut butter, honey, prune juice/grape juice, wine, and beer. In addition, the volunteers were advised to check labels and avoid products containing boric acid or sodium borate such as health food supplements, pharmaceuticals, skin ointments, cosmetics, and mouthwash or dental solutions. The volunteers were also asked to keep a detailed food diary of all of the foods and beverages they consumed for each of the 17 days during the study. In each daily record, the volunteer showed the time food/beverage was consumed, the location where it was consumed, type of food or beverage, method of food preparation, all ingredients, estimated amount eaten, and standard household measurements or the weight in ounces or grams.

**In vitro study.** Human cadaver skin was obtained from the Northern California Transplant Bank where tissues are tested for biosafety. Skin samples were handled, transported, and stored according to Wester et al. (1998) to maintain viability. The samples were not frozen or heat treated which destroys viability. Skin samples were visually inspected prior to study and observed for potential system leaks during the study. Phosphate-buffered saline (PBS) was chosen as the receptor fluid to avoid any possible boron analytical problems. PBS does not contain glucose and supportive chemicals, so in situ viability was not monitored. Skin sample 1 was thick skin from a 50-year-old male. Skin sample 2 was thick skin from a 51-year-old male. Skin sample 3 was thick skin from a 63-year-old female. Skin sample 4 was skin from a 39-year-old male. Skin sample 5 was thick skin from a 52-year-old male. Skin sample 6 was thick skin from a 65-year-old female. Prior to use, all skin was dermatomed using a Padgett Electrodermatome to a targeted thickness of 500 μm. Upon receipt, each skin sample was placed in Eagle’s minimum essential medicam (MEM) and stored at 1 ± 4°C until use within 5 days of death. This preservation/use regimen follows that used by the human skin transplant bank (Hurst et al., 1984) and the work of Bronaugh et al. (1989) and has been shown to keep human skin viable (Wester et al., 1998).

Dermatomed human cadaver skin was clamped onto an AMIE Systems in-line cell in a flowthrough diffusion cell apparatus. ([10B])Boron-enriched boric acid, borax, and disodium octaborate tetrahydrate in water solution were applied to the donor side of human skin mounted in teflon diffusion cells of flowthrough design. Teflon was chosen to avoid possible boron contamination from glass. The flowthrough design delivers a continual perfusion (3 ml/h) underneath the mounted skin, with mixing aided by means of a magnetic stirrer. The Teflon cells had a 1-cm² surface area of exposed epidermal skin, which was maintained at approximately 32°C using a Lauda heating circulator. The receptor fluid, fresh 0.01 M phosphate-buffered saline, was pumped at a rate of 3 ml/h. Receptor fluid was collected every 4 h to 24 h using a Retriever IV fraction collector. Twenty-four hours after dose application, skin surface wash was performed on all skin samples (washed once with Nanopure water, once with 1% Ivory liquid soap/Nanopure water, and again with Nanopure water).

**Sample analysis.** Urine samples were diluted 1:10 in 1% nitric acid for analysis. Either saline samples were diluted or, for low boron levels, 2 ml was passed through a strong cation exchange cartridge (J. T. Baker SCX-SPE, 3 ml) to lower the sodium concentration which caused signal suppression (Na > 500 ppm). Soap wash solutions were diluted 1:1000. Beryllium (100 μg/l) was added to all samples and standards either on line during analysis (for small sample volumes, 10 ml) or off line (for larger sample volumes). Skin tissue washes were analyzed for boron content by inductively coupled plasma-mass spectrometry (ICPMS) (Hauk and Thompson, 1988; Smith et al., 1991) using a VG PlasmaQuad II (VG Elemental, Danvers, MA). Samples were delivered by peristaltic pump (Gilson Minipuls 3, Middleton, WI) at approximately 1.2 ml/min. SRM 951, boric acid from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) which is certified for both purity and isotopic content, was used to prepare boron standards in the range of 10–1000 μg/L. The acid concentration in the standards was matched to the sample for accurate quantitation.

Below are listed typical instrument parameters for data acquisition. Boron background and carryover was controlled by limiting the sample size (approximately 0.4 ml) and concentration (<1000 μg/L). Samples (approximately 0.3 ml) were flow injected by controlling the time the autosampler (Gilson Model 222) was positioned in the sample tube to 20 s. Acquisition was timed for 10 s in the middle of the 20-s sample flow plug.
FIG. 1. Daily plot of excess boron 10 in urine for in vivo group topically dosed with $^{10}$B boric acid on days 5 and 12. Individual and mean values are shown.

Using these parameters (Table 1), the ratio of $^{11}$B/$^{10}$B was determined with a relative standard deviation of 1–2%. Using isotope ratios, the detection limit (99% confidence) for $^{10}$B spikes, in counts per second, in urine containing approximately 120 μg/L total boron was approximately 7 μg/L. The detection limit for $^{10}$B and $^{11}$B in water using an external standard calculation was approximately 0.2 and 1 μg/L, respectively. The quantitation limit is $3 \times$ the detection limit. Instrument background equivalent concentrations were generally less than 1 μg/L for total boron.

Statistical methods. The dietary mole ratio of $^{10}$B/$^{11}$B, which we call MRATIO, was determined separately for each subject by computing a weighted average of the $^{11}$B/$^{10}$B ratios from days 1–4, using the daily boron consumption as the weight. To obtain the dietary weight ratio of $^{10}$B/$^{11}$B, which we call WTRATIO, from the formula

$$WTRATIO = MRATIO \times \frac{11.0009305}{10.012937},$$

where the numeric ratio is the molecular weight of $^{10}$B/$^{11}$B. Then, the expected dietary level of $^{10}$B, EXPECT10, is given by multiplying the total daily boron level by $1/(1 + WTRATIO)$. To find the amount of $^{10}$B on days 5–17, referred to as ACTUAL10, the same procedure is followed, except that the laboratory ratio for the given day is used instead of MRATIO. If any $^{10}$B is absorbed through the skin, this ratio used should be lower than MRATIO. Thus, the amount of $^{10}$B obtained on days 5 through 17 should be as high or higher than EXPECT10. The amount of $^{10}$B absorbed through the skin, EXCESS10, is equal to ACTUAL10 – EXPECT10. Due to laboratory measurement errors and possible small changes in dietary $^{11}$B/$^{10}$B ratios on different days, it is possible for EXCESS10 to be negative.

Confidence intervals for the percentage of dose ($^{10}$B) absorbed for the three compounds were constructed using the $t$ distribution, namely, mean ± 2.365 × SD, where $t_{23.7} = 2.365$, where 0.025 = α/2 and 7 df. Comparison of percentage of boron absorption between compounds was assessed using analysis of variance. A logarithmic transformation was applied to the in vitro data to yield more Gaussian-like data for all analyses. The geometric means given are back transformations of standard means obtained using the log-transformed data.

For each solution–skin sample combination, boron fractions of the in vitro receptor fluid were obtained every 4 h over a 24-h period with a final fraction (RF), except for skin sample 4, where single 12- to 24-h fractions were obtained. Generally, the fraction amounts first increase and then decrease. In a few cases, dramatic jumps or drops were seen that were suspected of being gross errors. For example, the successive fractions for the percutaneous absorption of 0.05% at 1000 μl · cm$^2$ boric acid for skin sample 5 were 0.010 (0–4 h), 0.009, 0.004, 0.004, 0.004, 0.224 (20–24 h), and 0.004 (RF), with the 20- to 24-h value being 56 times the nearby fractions. To clearly identify the outliers in these data, ratios of successive 4-h fractions were displayed using boxplots (see Chapter 3 of Hoaglin et al., 1983) using the Statistical Analysis System SAS 6.11 (Cary, NC). Extreme outliers were identified by asterisks in the plots. All outliers identified using this method were judged to be gross data errors. New values for these outliers were imputed using least squares estimation from a linear model (Little and Rubin, 1987), where the log of the ratio of each successive fraction was modeled as a linear function of the corresponding solution and skin sample. Imputed values were then substituted for the outliers (in the example described above, 0.0056 was imputed for 20–24 h).

RESULTS

Figures 1–3 show the in vivo daily plot of excess $^{10}$B as individual and mean values for boric acid, borax, and DOT, respectively. Increased boron excretion can be seen following $^{10}$B-enriched dosing occurring on day 5 and on day 12 following pretreatment with 2% SLS.

Table 2 shows that 0.226 (SD = 0.125) mean percentage of dose of 5% boric acid was absorbed through the skin in human volunteers over a 6-day period after a 24-h dosing period. Pretreatment with 2% SLS for 24 h gave no change in mean percentage of dose absorbed (mean = 0.239, SD = 0.174). With 5% borax, the mean percentage of dose absorbed was
0.210 (SD = 0.194) with no treatment, and no change was seen with 2% SLS pretreatment (mean = 0.184, SD = 0.219). With 10% DOT, the mean percentage of dose absorbed was 0.122 (SD = 0.108) with no treatment, and no change was noted with 2% SLS pretreatment (mean = 0.107, SD = 0.133). No statistically significant difference was found in the percentage of boron absorption of the three compounds, either without pretreatment ($P = 0.33$) or after 2% SLS pretreatment ($P = 0.35$), even though the mean percentage of boron absorption of DOT was about half of that obtained for both boric acid and borax. No skin irritation was noted for any of the above treatments.

Table 3 gives the percutaneous absorption parameters for boron.
BORON HUMAN TOPICAL BIOAVAILABILITY

In Vivo Percutaneous Absorption of $^{10}$Boron as 5% Boric Acid, 5% Borax, and 10% Disodium Octaborate Tetrahydrate (DOT) in Normal Human Volunteers

Mean percentage of dose absorbed

<table>
<thead>
<tr>
<th>Compound</th>
<th>No pretreatment</th>
<th>2% SLS pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>5% boric acid</td>
<td>0.226</td>
<td>0.125</td>
</tr>
<tr>
<td>5% borax</td>
<td>0.210</td>
<td>0.194</td>
</tr>
<tr>
<td>10% DOT</td>
<td>0.122</td>
<td>0.108</td>
</tr>
</tbody>
</table>

* n = 8; 24-h dosing period.

* 95% confidence interval for the mean based on the t distribution.

* Paired t test p value for the difference in mean percentage of dose absorbed.

boric acid, borax, and DOT. Flux is the rate of absorption ($\mu g/cm^2/h$), and the permeability constant $K_p (cm \times h^{-1})$ can be used to calculate absorption for any topical dose. Since 5% boric acid, 5% borax, and 10% DOT doses are at near chemical solubility limits, these absorption parameters can be considered to be at maximum value. The absorption parameters of all three boron containing compounds, boric acid, borax, and DOT, can be considered low compared to other chemicals.

Table 4 gives the boron recovery and accountability of the administered dose in the 24-h urine accumulation (percentage of dose absorbed), in the T-shirt that was worn over the dosed skin for 24 h, and from the skin washings at the end of the 24-h period. T-shirts were worn to reduce transfer of the applied dose, after drying, to outside clothing or bed clothes during the 24-h absorption period. As can be seen from Table 4, approximately 50% of the applied dose was extracted from the T-shirt at the end of the absorption period. Some 5-10% still remained on the skin. The remainder of the applied dose probably dusted off into the air or rubbed off onto other clothing rather than being retained as would have been the case had an occlusive bandage been used. Thus approximately 60% of the applied dose is lost (Wester et al., 1992, 1993).

Table 5 gives the in vitro percutaneous absorption in human skin of boron when dosed as boric acid, borax, and DOT in water. An analysis of variance (ANOVA) on total boron absorbed for each solution–skin sample was conducted. The statistical model provided a very good fit to the data ($r = 0.88$). There was a difference in the absorption between skin samples. The average percentages of boron absorption for the six dosing solutions were 2.0, 0.30, 1.7, 4.6, 0.05, and 0.14, for skin sources 1 through 6, respectively.

Boric acid was dosed at three different concentrations (5, 0.5, 0.05, w/v) at 1000 $\mu l/cm^2$. The dose response is shown in Fig. 4. Flux ($\mu g/cm^2/h$) increased with increased concentration, and the permeability constant (cm/h X $10^{-4}$) did remain relatively constant. Therefore, the higher the concentration the more mass that will be absorbed. After 24 h, the remaining dose was removed and the skin was washed with soap and water. Most of the dose was accounted for in these washes (72.4 ± 9.1, 86.0 ± 5.9, and 81.9 ± 2.9% for 0.05, 0.5, and 5.0% doses, respectively).

Boric acid at 5% concentration was dosed at a traditional in vitro 1000 $\mu l/cm^2$ volume and at a volume relevant to the in vivo study at 2 $\mu l/cm^2$ (higher volumes in vivo would just run off the skin). More mass was absorbed at the higher volume because more mass was available to be absorbed. At the lower volume, some 200X less boric acid was absorbed (14.58 versus 0.07 $\mu g/cm^2/h$).

Boric acid, borax and DOT were dosed at 5, 5, and 10%, respectively, all at 1000 $\mu l/cm^2$ (Table 5). $K_p$ of boron permeability is greatest for boric acid (2.9 X $10^{-4}$ cm/h), then borax (1.7 X $10^{-4}$ cm/h), and then DOT (0.8 X $10^{-4}$ cm/h).

TWEAL measurements showed no difference between untreated skin and that treated with 2% SLS. Water loss (g/m$^2$/h) for boric acid was 9.2 ± 2.6 pre-SLS and 7.5 ± 0.8 post-SLS ($p = 0.1$), for borax it was 6.9 ± 1.5 pre-SLS and 6.9 ± 1.3...
TABLE 4
Dose Accountability for $^{10}$Boron as 5% Boric Acid, 5% Borax, and 10% Disodium Octaborate Tetrahydrate (DOT) in Normal Human Volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5% boric acid</th>
<th>5% borax</th>
<th>10% DOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>%- of dose absorbed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>0.226 (0.125)</td>
<td>0.210 (0.194)</td>
<td>0.122 (0.108)</td>
</tr>
<tr>
<td>SLS treatment</td>
<td>0.239 (0.174)</td>
<td>0.185 (0.219)</td>
<td>0.107 (0.133)</td>
</tr>
<tr>
<td>T-shirt (24 h)</td>
<td>50.9 (16.6)</td>
<td>47.4 (12.4)</td>
<td>57.2 (11.0)</td>
</tr>
<tr>
<td>No treatment</td>
<td>53.3 (5.9)</td>
<td>51.2 (4.0)</td>
<td>43.8 (26.5)</td>
</tr>
<tr>
<td>Skin wash (Nos. 1–9)</td>
<td>6.1 (1.8)</td>
<td>10.6 (1.8)</td>
<td>6.0 (3.6)</td>
</tr>
<tr>
<td>SLS treatment</td>
<td>6.9 (4.1)</td>
<td>4.6 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Skin wash (No. 10)</td>
<td>0.4 (0.40)</td>
<td>0.9 (0.25)</td>
<td>0.3 (0.15)</td>
</tr>
<tr>
<td>SLS treatment</td>
<td>0.3 (0.13)</td>
<td>0.7 (0.26)</td>
<td>0.3 (0.22)</td>
</tr>
</tbody>
</table>

DISCUSSION

The undertaking of this study presented a challenge not frequently encountered by those who attempt to measure percutaneous absorption of chemical substances. In this study of borates with no radioactive label available, we had to measure small amounts of skin-absorbed boron in the presence of a relatively large amount of normal dietary boron. This was made possible by the existence of two stable isotope forms of boron, $^{10}$B and $^{11}$B, measurable by recently available analytic technology. The study was also made possible by the fact that, in humans, boron is excreted into the urine rapidly and nearly completely, 90–100%, so that any bone deposition must be very small and would not change the estimated percentage of boron absorbed through the skin by a measurable amount. During the study days 5 through 17 that followed the skin application of the test dose, the average daily dietary boron consumed by each subject was 1750 /μg. Given the dietary ratio of $^{10}$B/$^{11}$B established from the baseline data collected during the study days 1 through 4, we estimate the dietary $^{10}$B consumption to be 316 /μg. The average boron (all applied boron was $^{10}$B) level absorbed through the skin for days 5–17 from all subjects was 4.75 /μg. Thus, the average amount of boron absorbed through the skin represents 0.27% of the total boron and 1.5% of the $^{10}$B obtained from urine specimens, respectively.

Since the design of this study required analytic results that were close to the edge of current boron analysis technology, there was a need to determine whether the results had some credibility. The study design, in which each subject was dosed twice and absorption was measured twice, allowed us to do paired comparison of the absorption results. There was a strong correlation ($r = 0.92; p = 0.001$) between subjects’ mean dose absorption for DOT with and without SLS pretreatment. The correlations for boric acid ($r = 0.69; p = 0.07$) and borax ($r = 0.57; p = 0.14$) were both moderately positive although not statistically significant. These comparisons give us a measure of the success of the methods used. The percentage of dose absorptions shown in Table 1 are small, but all of them do demonstrate statistically some boron absorption through the skin, except for DOT following SLS pretreatment (where the 95% confidence interval includes zero).

In the clinical study, percutaneous absorption of boron, as boric acid, borax, or DOT, was determined in eight human volunteers for each dose group (Table 1). The intact skin absorption results were compared with those from SLS treatment (Table 2). Although SLS is known as a human skin irritant, measurable irritation was not obtained in this study.

TABLE 5
In Vitro Percutaneous Absorption of Boron Administered as Boric Acid, Borax, and Disodium Octaborate Tetrahydrate (DOT) in Human Skin

<table>
<thead>
<tr>
<th>Dosing solution</th>
<th>Percentage of dose absorbed geometric mean (95% Cl)</th>
<th>Flux (μg/cm²/h)</th>
<th>$K_p$ (cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boric acid (w/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% at 2 μl/cm²</td>
<td>1.75 (0.18–17)</td>
<td>0.07</td>
<td>$1.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>5% at 1000 μl/cm²</td>
<td>0.70 (0.072–6.81)</td>
<td>14.58</td>
<td>$2.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>0.5% at 1000 μl/cm²</td>
<td>0.28 (0.029–2.72)</td>
<td>0.58</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>0.05% at 1000 μl/cm²</td>
<td>1.20 (0.012–11.7)</td>
<td>0.25</td>
<td>$5.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>Borax (w/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% at 1000 μl/cm²</td>
<td>0.41 (0.042–3.99)</td>
<td>8.5</td>
<td>$1.7 \times 10^{-4}$</td>
</tr>
<tr>
<td>DOT (w/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% at 1000 μl/cm²</td>
<td>0.19 (0.018–1.81)</td>
<td>7.9</td>
<td>$0.8 \times 10^{-4}$</td>
</tr>
</tbody>
</table>
and absorption of boric acid and inorganic following SLS treatment did not significantly differ. The in vivo results show that percutaneous absorption of boron, as boric acid, borax, and disodium octaborate tetrahydrate, is low. The range for the borates in vivo absorption was 0.1–0.2% applied dose. This is less than the metals arsenic (2%), cadmium (7%), mercury (29%), or a variety of organic chemicals (Wester and Maibach, 1996).

Percentage of absorption information from this study can be used to estimate absorbed dose from cutaneous exposure. For a worst-case analysis one could take as an example the wetting of the entire skin surface with a saturated solution of boric acid (approximately 5%), allowing it to dry, and remain on the surface for 24 h. Experience in our laboratory has been that for low viscosity liquids, the maximum volume that will remain on human skin surfaces without running off is 2 µl/cm². Thus, for a “standard” 1.8-m² man, the volume of solution that could be applied to the whole body surface would be 36 ml or approximately 1.8 g of boric acid containing 315 mg of boron. Based on an absorption of 0.226% over a 24-h period, this worst-case estimate would amount to 0.7 mg per day. This can be compared with mean dietary boron intake by 24 subjects during the 4-day baseline period of 1.75 mg per day (range of 0.163 to 8.70 mg) estimated from 24-h urine samples. The worst-case estimate of 0.7 mg B per day is also less than the reported NAOEL of 9.6 mg B/kg/day by a factor of more than 800 for the most sensitive effect (Price et al., 1996).

Recovery of the in vivo dose 24 h after skin application was 44–57% of applied dose in the T-shirts and 5–11% in the skin washes. This shows that dose was on the skin during the entire dosing period. This is excellent for a walk-around human subject where daily activities and sleep can lose dose to a variety of situations (Wester et al., 1992, 1993).

From this study, it is apparent that the wearing of gloves for the prevention of skin absorption in borate workers is unnecessary. With regard to abraded skin, or in the presence of dermatitis, it is unlikely that our absorption figures will apply. The estimates given here of the amount of solution that can be retained on skin surface and, together with the area of skin damage, can perhaps provide an upper bound on the amount of material available for absorption.

In the in vitro study, doses were administered as infinite (1 ml/cm²) or finite (2 µl/cm²) amounts. The infinite dose provides an unlimited amount of chemical available for absorption throughout the entire dosing period. The finite dose is that dose which would, in a practical sense, remain on the skin in an in vivo situation (the 1 ml/cm² dose would run off the skin, leaving about 2 µl/cm² remaining). The corresponding in vivo study was done using a 2 µl/cm² dose. Because of the known absorption variability in skin samples both in vitro (Wester and Maibach, 1991) and in vivo (Wester and Maibach, 1985), this in vitro study was done with skin from six different human cadavers. The variability seen here is within the expected range for in vitro studies.

Boric acid was dosed at 0.05, 0.5, and 5% at 1 ml/cm² (Table 4). The flux increased with concentration, as expected. Other studies also show that increased mass is absorbed with higher concentrations (Wester and Maibach, 1989). These results were obtained with an infinite dose (1 ml/cm²) so extrapolation to a finite dose must be done with caution. The permeability constant $k_p$ which normalizes absorption did not differ much by comparison (Fig. 1), and this occurred, ranging from 1.2 to
The only exception might be where a subject bathed in a boric acid, Borax and DOT were dosed at 5% (borax) and 10% (DOT) concentration (approximate saturated solution) at 1 ml/cm², an infinite dose. Flux and \( K_p \) values were in the range of boric acid when dosed at the higher dose and infinite supply.

Most of the \textit{in vitro} dose was recovered in the 24-h soap and water wash, with percentage of dose recoveries between 82 and 91%.

Table 6 compares the \textit{in vitro} absorption data from this study with data from the corresponding \textit{in vivo} study. The \( K_p \) values from the infinite dose are a 1000-fold higher than from the \textit{in vivo} study, while the finite dose \( K_p \) of boric acid was only 10-fold higher. Clearly, use of a similar dose volume was critical to the comparison. The \textit{in vitro} system used a phosphate-buffered saline perfusion as well as a water solution. The \textit{in vivo} dose was allowed to dry, and absorption took place in whatever water content the skin of each volunteer contained. The preponderance of water may have influenced the skin membrane during 24-h continual exposure, and this may have increased the permeability of the borate chemicals. This would make the \textit{in vivo} data and the \textit{in vitro} finite dose data more relevant for the usual exposure conditions. The only exception might be where a subject bathed in a boric acid, borax, or DOT solution for an extended period of time. The \textit{in vitro} infinite dosing conditions may better predict the bathing situation. But it is important to remember that there was a 1000-fold difference between \textit{in vivo} absorption and \textit{in vitro} absorption where the \textit{in vitro} dose was infinite. General risk assessment from \textit{in vitro} infinite dosing may be greatly over-estimated.

**CONCLUSION**

\textit{In vivo} absorption of boron applied for 24 h to human skin as boric acid, borax, or disodium octaborate was in the range of 0.12–0.23% and did not vary significantly from one borate to the other. This is equivalent to 0.7 mg of absorbed boron for a person entirely immersed in a saturated boric acid solution for 24 h. For comparison, 0.7 mg B is significantly less than the average daily dietary intake. In the replication of the study with the pretreatment of skin with SLS prior to the application of borate, there was no detectable erythema, change in transepidermal water loss or effect on boron skin absorption. Thus the replication study using SLS provided an opportunity for confirmation of the results of the initial borate applications in which absorption ranged from 0.11 to 0.24%. This very low boron skin absorption makes it apparent that, for borates tested that have low human toxicity, the use of gloves to prevent systemic uptake is unnecessary. However, the findings of this study do not apply to abraded or otherwise damaged skin.

From the \textit{in vitro} part of this study, where the amount of borate available to the skin surface was not limited to the amount that could be kept in contact with the surface, larger amounts of boron could be absorbed. Whether this finding from an \textit{in vitro} model would be applicable to prolonged \textit{in vivo} immersion in a borate containing bath is speculative and will require further study. It is suggested that finite dosing be used and that infinite dosing be confined to limited use and only where warranted.

**REFERENCES**


