INVESTIGATIVE REPORT

# Human Stratum Corneum Adsorption of Nickel Salts
## Investigation of Depth Profiles by Tape Stripping *In Vivo*

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**Sequential adhesive tape stripping was implemented to characterize the penetration of nickel salts in human stratum corneum. Exposure areas of the salts in methanol applied open on arm and back skin in low volume were stripped 20 times to the level of the glistening layer at intervals of 30 min to 24 h post-dosing, and the strips analyzed for metal content by inductively coupled plasma–atomic emission spectroscopy. In the case of nickel chloride, sulfate, nitrate and acetate, material left on the skin surface, the depth-penetration profiles in the stratum corneum, and the dosage unaccounted for suggest the following conclusions: (a) Up to 24 h, most of the nickel dose applied remains on the skin surface or is adsorbed in the uppermost layers of the stratum corneum. (b) At higher concentrations, incomplete material recovery becomes discernible; within 24 h, nickel salts thus appear to penetrate beyond the stratum corneum to a minor degree, possibly via the skin shunts. (c) While the concentration gradients of nickel adsorbed vary with counter ion, anatomical site, dose and exposure time, for all variables tested the depth profiles converge to non-detectable levels ($< 20$ ppb) towards the level of the glistening layer. A notable exception is nickel as nitrate, for which levels continue at low but constant levels (1% of dose) beyond the third stratum corneum strip, indicative of intercellular diffusion. (d) Differences in material recovered suggest that the stratum corneum on the arm is more penetrable to nickel than stratum corneum on the back. (e) The counter ion in nickel salts plays a major part in their diffusion into the stratum corneum, suggestive of ion pairing. Overall, the data point to all three avenues of skin penetration by nickel: intracellular, intercellular, and transappendageal. *Key words: allergic contact dermatitis; human; inductively coupled plasma, atomic emission spectroscopy; in vivo; nickel; shunt diffusion; stratum corneum adsorption; tape stripping.***

Nickel is recognized as the premier cause of allergic contact dermatitis in the industrialized world. While naturally occurring nickel compounds are not immunogenic, anthropogenic nickel salts and alloys, as well as the metallic form of the element, have proved to be a health hazard with the advent of the industrial age. Nickel belongs to the group of metals which react with sweat and can form divalent nickel ions; these in turn can penetrate the stratum corneum (SC) via the appendageal, transcellular or intercellular route to reach the viable epidermis. Reacting there with amino acid residues the resulting nickel-complexed protein may then cause contact allergy, as well as irritation (1).

We sought to define the characteristics of diffusion of water-soluble nickel salts over 24 h throughout the microenvironment of the human SC. We closely examined nickel localization in the SC *in vivo*, since earlier investigations had raised a number of questions concerning the behavior of nickel in contact with human skin: pronounced allergenicity in spite of low diffusivity (2–6), dependence of skin permeability on counter ion (2, 3, 6, 7) and anatomical site (8), appendageal penetration (9–11), and the formation of depots in the skin (2, 9, 12–14). These observations prompted the investigation of depth profiles obtainable by the non-invasive method of SC stripping with adhesive tape. A closer scrutiny of the surface adsorption dynamics of nickel as prototype of strongly electrophilic metals and its behavior in contact with human skin, as may occur particularly in the work environment, could clarify some of the unresolved questions associated with the allergenicity and dermatotoxicity of that metal.

In the present context, the term "Stratum Corneum Adsorption" is used to indicate that part of the dose applied which is found in the SC; "Dermal Absorption" denotes the balance not recovered by decontamination and SC stripping, thus potentially becoming systemically available.

## MATERIALS AND METHODS

### Volunteers

Five non-atopic investigators (4 men, 1 woman; aged 30–62, mean $39 \pm 16$) in good health and with no history of allergies or significant skin disease participated in the study conducted over a period of 2 years. The study was approved by the UCSF Committee on Human Research; informed consent was obtained from all subjects. Repeat application of test solutions on the skin followed by stripping was avoided earlier than 30 days following previous stripping of the area. As a rule, replicate experiments for statistical purposes were conducted on the same volunteer to minimize experimental variability. Also for each of the parameters investigated, the stripping experiment was conducted on the same volunteer.

### Chemicals and materials

The nickel salts $NiCl_2 \times 6H_2O$, $NiSO_4 \times 6H_2O$, $Ni(NO_3)_2 \times 6H_2O$ and $(CH_3CO_2)_2Ni \times 4H_2O$, were best commercially available grades (Aldrich Chem. Co., Milwaukee, WI). Methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA), n-octanol from Sigma Chemicals (St. Louis, MO, USA), and conc. trace metal-grade nitric acid (70%) from EM Science, (Gibbstown, NJ, USA). Millipore Millex non-sterile syringe filters (Fischer Scientific) were used for filtration, polypropylene tape with a backing of pressure-sensitive acrylate adhesive (3M Transpore surgical tape of 1-inch width, 3M Health Care, St. Louis, MO, USA) was used for stripping.

*Reagent preparation and solubility tests*

Salts were dissolved in methanol targeting a nickel content of 0.001% to 1%. The actual nickel concentration in the test solutions was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) analysis, prior to application, to allow accurate interpretation of results. Methanol was chosen as a model vehicle with the intent of minimizing disruption of SC membrane integrity, of optimizing volatility and thus expediting open application, and for adequate solubility of nickel salts.

The solubility of nickel salts in n-octanol was determined by equilibrating excess solid compound through shaking in the solvent at room temperature over 72 h. After that time the slurries were centrifuged, the supernatant filtered through syringe filters of 0.2 μm pore size and the filtrate submitted for ICP-AES analysis.

*Skin application, decontamination and sequential stripping*

Prior to application of test solution, the targeted area of the skin was cleaned by threefold wiping using cotton swabs moistened in de-ionized water, then dried with a stream of compressed air for 30 sec; 100 μl of the solution was applied on a 2.83 cm$^2$ area of the skin using a shallow glass cylinder (1.9 cm inner diameter) for containment, to the base of which a film of silicone grease had been applied to prevent leakage. Following application of the test solution, a gentle air stream (compressed air) was directed at the application site through a plastic tube to enhance solvent evaporation, usually complete within 60 sec. Throughout the experiment, care was taken not to touch the area of application; in the case of exposure times of longer than 3 h, the application sites were covered with a rigid, perforated plastic shield open at both ends to ensure free air circulation but at the same time prevent mechanical abrasion. The shield was held in place with tape, the tape strips being applied transversally across the shield in such a way as not to cover the air vents. At the end of the dosing period, in several experiments the application sites were wiped clean with cotton swabs to remove residual test material remaining on the surface prior to tape stripping. Repeated wiping with a water-moistened cotton swab was followed by a dry swab, and finally an air stream was passed over the skin surface for 30 sec. The combined swabs were placed in a glass vial for separate extraction and nickel analysis.

Adhesive tape (2.54 cm width) was cut into 5 cm strips, and the SC stripped in accordance with the following protocol. The area of application on the volar forearm between cubital fossa and wrist or on the intrascapular area on the back was marked using a felt-tipped marker pen, then stripped sequentially 20 times. This was accomplished by covering the 2.83 cm$^2$ treated with the pre-cut tape, thus removing SC from a 6.45 cm$^2$ area, abundantly exceeding the area originally exposed in dosing. Constant and uniform pressure (100 g/cm$^2$) was applied on the tape for 5 sec by resting an appropriate weight on the area, and the tape then gradually removed from the skin in one draw. Each tape strip was stored individually in a 20-ml glass vial.

Five milliliters of concentrated nitric acid was added to the vials containing tape strips and decontamination swabs. After 3 h of vigorous agitation in a rotary shaker (Gyrotory water bath, model G76; Edison, NJ, USA), the acid solution was diluted to 10 ml with de-ionized water prior to analysis by ICP-AES.

For the purpose of cost control, of the total 20 strips taken per exposure an average of 10 were submitted for analysis at intervals appropriate to yield an adequate curve. For the purpose of total dose recovery determination, all 20 strips were analyzed from a limited number of experiments.

*Controls*

For negative control, all reagents and materials, including acid, water, marker ink and skin strips taken in triplicate from the non-exposed area on the same skin region of the respective investigator, were analyzed for nickel content at the time of the experiment. All values were below nickel detection limits (< 20 ppb). For positive control, all nickel salt solutions applied were checked for actual nickel content by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

*Method of analysis and nickel detection limit*

Analysis was performed at the University of California (Berkeley) Microanalytical Laboratory. All nickel concentrations were determined on a Perkin Elmer Optima 3000DV ICP-AE spectrophotometer. The detection limit for nickel using the ICP-AES method was set at 20 ppb, defined as three standard deviations above the instrument's detection limit of 7 ppb.

*Statistical analyses*

Statistical analyses of the data were performed using Student's paired *t*-test on MS Excel and Microcal ORIGIN. The probability value $p < 0.05$ was considered significant. Area under the curve (AUC) to the end point of measurement (cumulative amount of SC up to 20 strips) was calculated using Microcal ORIGIN.

## RESULTS

*Recovery of nickel dose applied*

Nickel recovery by skin surface decontamination prior to stripping, and by strip analysis following open application, is presented in Table I, grouped according to the recovery method used. From surface decontamination values (Table I) certain trends become apparent indicative of SC adsorption of nickel in function of time, dose, site and counter ion. Total recovery calculated as percent of dose (Table I) is virtually quantitative for most experimental parameters. Only at the highest concentration applied as nickel nitrate does potential absorption beyond the SC become discernible through incomplete material balance.

*Dependence of adsorption on dose*

Nickel retained in the outer SC layers increases with rising concentration applied, but beyond strip No. 15 at all concentrations the levels adsorbed converge towards the limit of detection ($n = 3$). The change in initial concentration gradients as a function of concentration is illustrated for nickel chloride in Fig. 1.

*Adsorption in function of exposure time*

Commensurate with increasing time of exposure, nickel accumulates in the outer layers of the SC. Concentration gradients as a function of time are illustrated for nickel chloride in Fig. 2 ($n = 3$). Amounts recovered by stripping decrease with depth and approach the detection limit (20 ppb) beyond strip No. 15, independently of exposure time.

*Intra-regional skin differences in adsorption derived from areas under the curve (AUC)*

Nickel recovered with SC strips following application as chloride on the back (AUC = 30.8 ± 1.3; $n = 3$) is significantly less ($p = < 0.0005$) than nickel removed from the arm of the same volunteer (AUC = 62.4 ± 0.9; $n = 3$) at the same dose and exposure time. At both sites the amounts converge towards non-detectable levels beyond the 15th strip (Fig. 3).

*Effect of counter ion on adsorption*

Applied as four salts on the arm at an approximate concentration of 0.1%, nickel is adsorbed at different rates as evident

Table I. *Skin surface decontamination and total recovery values for nickel salts*

| Nickel salt | Dose ($\mu g/cm^2$)/site | Time | Nickel on skin surface (% dose) | Total recovery (% dose) |
|---|---|---|---|---|
| Chloride | 19.8/arm | 30 min | $89 \pm 10.4$ ($n = 5$) | |
| | 19.8/arm | 3 h | $84 \pm 7.1$ ($n = 2$) | |
| | 19.8/arm | 12 h | $75 \pm 6.7$ ($n = 2$) | |
| | 19.8/arm | 24 h | $58 \pm 6.1$ ($n = 3$) | $99.7 \pm 3.0$ ($n = 3$) |
| | 1.8/arm | 24 h | $94 \pm 7$ ($n = 2$) | |
| | 314/arm | 30 min | $54 \pm 7.1$ ($n = 5$) | |
| | 314/back | 24 h | $51 \pm 6.3$ ($n = 3$) | $103.9 \pm 4.8$ ($n = 2$) |
| Nitrate | 38.5/back | 24 h | $42 \pm 6.9$ ($n = 2$) | $97.3 \pm 2.8$ ($n = 2$) |
| | 357/back | 24 h | $30.5 \pm 5.5$ ($n = 2$) | $87.5 \pm 11.8$ ($n = 2$) |
| | 357/arm | 24 h | $27.9 \pm 2.6$ ($n = 2$) | $72.0 \pm 3.0$ ($n = 2$) |
| Sulfate | 37.1/arm | 30 min | $89 \pm 9.9$ ($n = 3$) | $98.6 \pm 3.6$ ($n = 3$) |
| | 37.1/back | 24 h | $52.7 \pm 8.1$ ($n = 2$) | $88.6 \pm 7.0$ ($n = 2$) |
| Acetate | 56.1/arm | 30 min | $90.1 \pm 7.3$ ($n = 2$) | $97 \pm 4.3$ ($n = 2$) |

Analysis for residual nickel on the skin surface, collected by decontamination through swabbing prior to stripping, and nickel recovery from analysis of strip nos. 1–20, collected without prior surface decontamination.
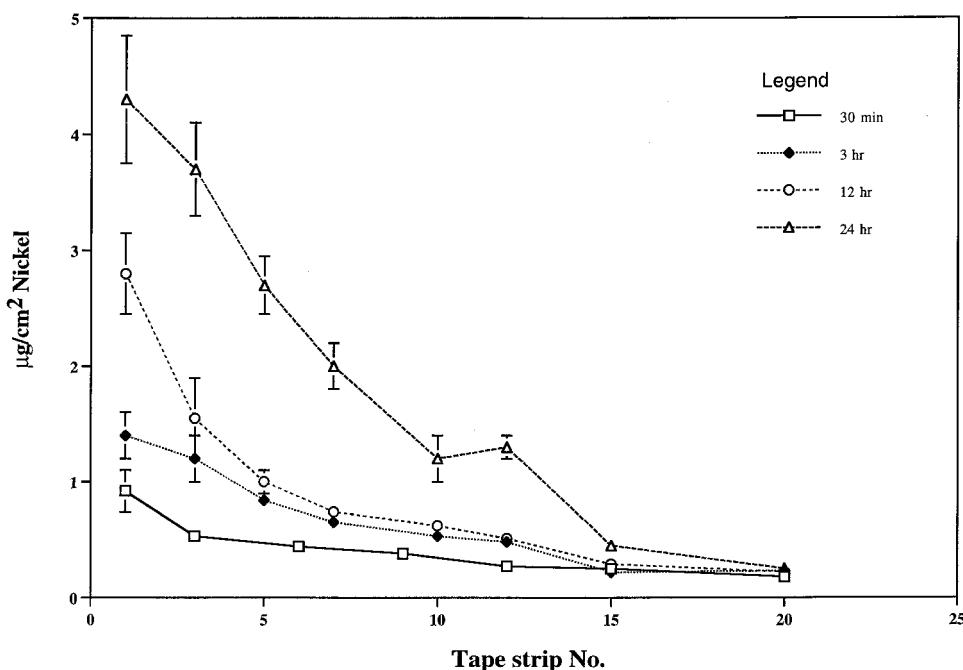


*Fig. 1.* Depth profile of nickel in stratum corneum in function of exposure time. $NiCl_2$ in methanol applied on the arm at $19.8 \, \mu g/cm^2$ Ni(II) ($n = 3$); site stripped after surface decontamination. Areas under the curve (AUC) for 30 min, 12 h and 24 h (6.9, 13.8, 20.7) are significantly different from each other.

from strip analyses at 30 min post-dosing (Fig. 4). While depth profiles converge towards non-detectable levels in the lower strata for the acetate, chloride and sulfate, nickel applied as nitrate is retained in the SC at a constant level of approximately 1% of applied dose beyond strip No. 3 through No. 20. AUC for the nitrate is significantly greater than for the other salts ($p < 0.05$).

*Polarity of nickel salts*

The polarity of nickel salts as measured by their solubility in the non-polar solvent n-octanol at 22°C increases in highly significant intervals ($p < 0.0005$) in the sequence: nitrate < chloride < acetate < sulfate, as presented in Table II.

DISCUSSION

The principal aim of this study was to investigate the diffusion of water-soluble nickel salts through the SC following skin contact, and by inference assess the amount which will reach the viable epidermis. We also anticipated that results from this investigation would explain some of the seemingly paradoxical observations made for the pharmacodynamics of nickel in earlier investigations.

To obtain an accurate, quantitative concentration profile of a skin permeant, analytical results would need to be normalized for the mass of SC removed with each individual tape strip. However, while mean thickness and number of cell layers of the human SC vary inter- and intra-individually with anatomical site, investigations using different adhesive tapes (Scotch
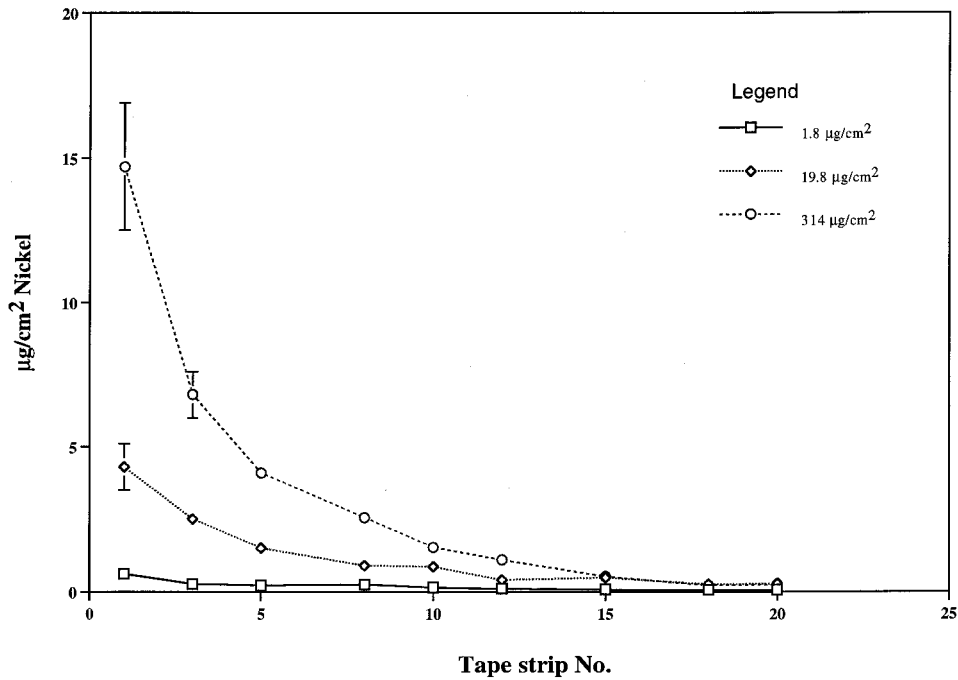
*Fig. 2.* Depth profile of nickel in stratum corneum in function of concentration. NiCl$_2$ in methanol applied on the arm ($n = 3$) over 24 h; site stripped after surface decontamination. Areas under the curve (AUC) for the three concentrations (3.4, 20.4, 53.1) are significantly different from each other.
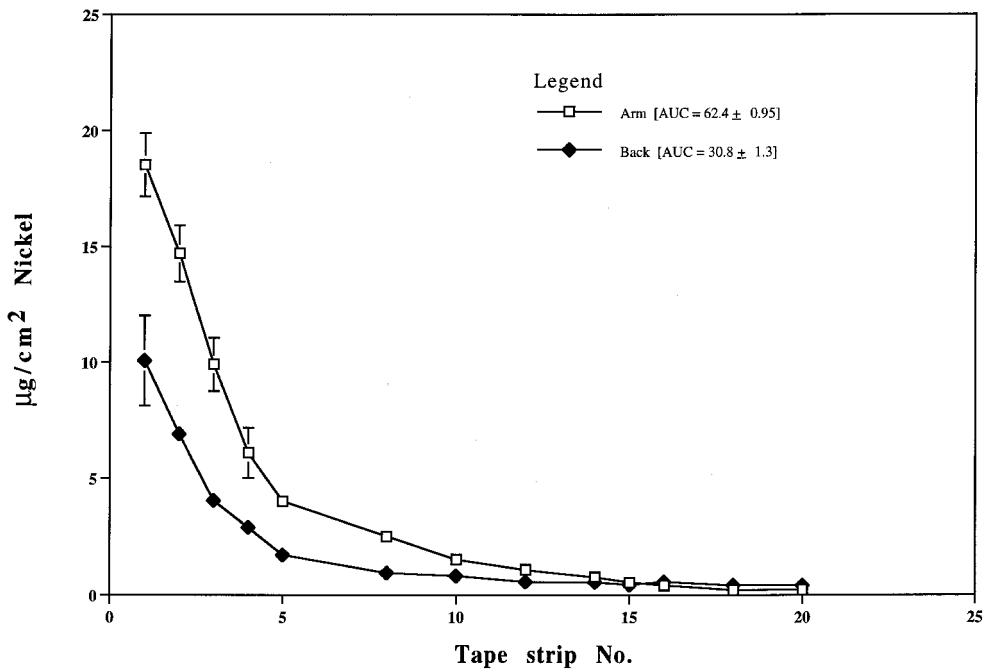


*Fig. 3.* Depth profile of nickel in stratum corneum in function of anatomical region. NiCl$_2$ in methanol applied on the arm and back over 24 h at 314 µg/cm$^2$ Ni(II) ($n = 3$); site of application was stripped after surface decontamination. Areas under the curve (AUC) are significantly different for the two sites (30.8 vs. 62.4; $p \leq 0.0005$).

book tape; D-Squame disks; Transpore tape), and different methods of analysis, such as gravimetry (15–17), attenuated-total-reflectance Fourier-transform infrared spectroscopy (18) and colorimetry (19), demonstrated that, after the first two to three strips, for a given skin site and test subject each tape strip removed a constant amount of SC. The number of tape strips strongly correlates with the cumulative SC mass removed, to approximately the twentieth strip (correlation coefficients r$^2$ = 0.95–0.99 (17). This then allows establishing a reliable permeant concentration profile in function of strip number for the interval 3rd to 20th strip, directly from analytical values.
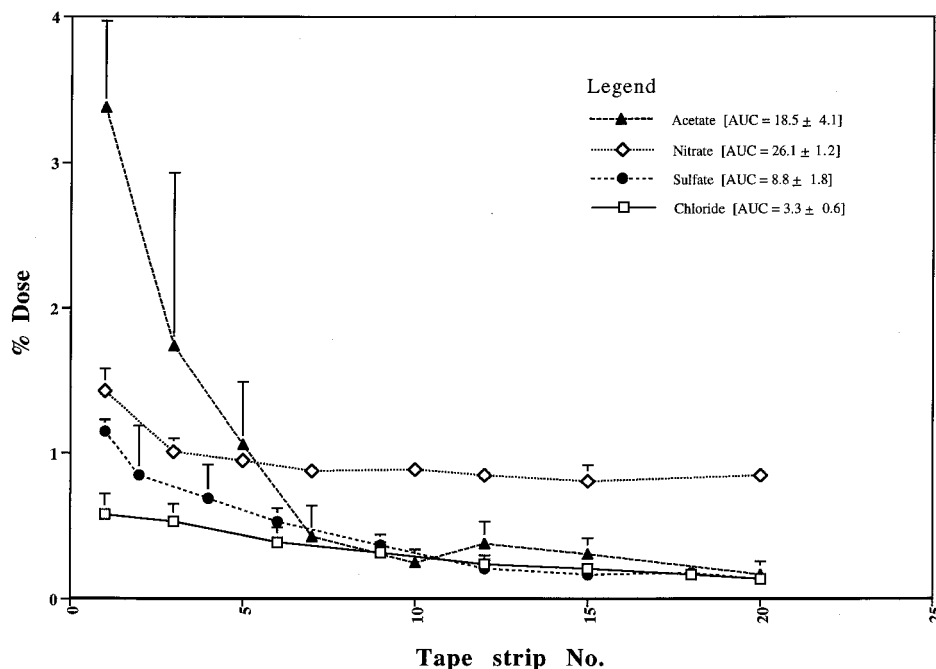
*Fig. 4.* Depth profile of nickel in stratum corneum in function of four counter ions. Nickel salts in methanol applied on the arm ($n = 3$) over 30 min at approximately 1% Ni(II) concentration and site stripped after surface decontamination. Area under the curve (AUC) for nitrate is significantly greater than for the other salts ($p < 0.05$).

Table II. *Solubility of nickel salts in n-octanol and amount adsorbed in the stratum corneum as area under the curve (AUC)*

|  | Chloride | Nitrate | Sulfate | Acetate |
|---|---|---|---|---|
| Solubility (ppm Ni; $n = 3$) | 11,366 | 24,283 | 2.07 | 1,822 |
| SD | 253 | 29 | 0.67 | 51 |
| AUC (% of dose; $n = 3$) | 3.3 | 26.1 | 8.8 | 18.5 |
| SD | 0.6 | 1.2 | 1.8 | 4.1 |

The solubility of each salt in n-octanol is significantly different from that of the others ($p < 0.0005$).
Area under the curve (AUC) is nickel recovered from 20 SC strips after surface decontamination. AUC is greatest for nickel nitrate ($26.1 \pm 1.2$), the most lipophilic of the four salts.

One earlier experiment appears particularly relevant as a benchmark for comparison with our results. To analyze for nickel depth profiles, the stripping method had been used *in vitro* on human full thickness skin following exposure by means of diffusion cells (12). Depth profiles in that study showed that following 96 h exposure to 5% nickel chloride in a 5% methyl cellulose gel, 51% was present in the SC, 11% in the epidermis, 2% in the dermis, and only 0.4% reached the receptor solution. Total nickel accounted for in that experiment was 64% of the applied dose.

In our study, material recovered post-dosing by skin surface decontamination and that recovered by sequential SC strip analysis notably confirms the limited potential for percutaneous absorption of nickel ion, as well as a pronounced variability in its retention in the SC. At lower doses, based on cumulative values of strip nos. 1–20, recovery of nickel applied up to 24 h appears virtually quantitative, although potential differences may escape detection in that range because of the magnitude of experimental error. The data presented in Table I, categorized by counter ion, demonstrate the effectiveness of the SC barrier against significant percutaneous absorption by charged molecules, of highly electrophilic metals

such as nickel in particular. It also allows some conclusions as to the quantitative behavior of the various nickel salts in contact with skin, and presents evidence for differential affinity of the nickel ion for protein, apparently moderated by the counter ion.

Measured at increasing intervals up to 24 h, percent of dose left unadsorbed on the skin surface decreases with increasing time of exposure and concentrations applied: decontamination values of chloride and sulfate from arm skin prior to stripping are significantly lower ($p < 0.05$) after 24 h as compared to 30 min exposure (Table I). The decrease in surface decontamination values is also significant going from lowest to highest chloride dose applied on arm skin ($p < 0.05$). This trend in disappearance values is intuitive for duration of exposure. Steeper gradients in nickel retained in superficial SC layers with increasing ion strength (Fig. 1) can be attributed to diffusion pressure as the force driving penetration. This is consistent with the observation *in vitro* that nickel skin permeation values increase by an order of magnitude corresponding to a similar increase in donor concentration (2). Similarly, depth profile gradients increase with exposure time (Fig. 2).

While percent dose left on the skin surface can vary

depending on experimental parameters (Table I), in most cases without prior surface decontamination all of the material applied is accounted for by SC strip analysis (Table I). Only at higher concentrations, at the level of 1% nickel, does incomplete material balance become measurable in our experiments, i.e. only relatively small percentages of the xenobiotic violate the SC barrier and penetrate the deeper layers. The fact that part of the applied dose remains unaccounted for even after SC levels taper off to insignificant levels is highly suggestive that nickel, like other metal ions, chooses the alternate, shunt pathway for diffusion and deposition in the deeper layers of the skin, as has been observed by others (9–11, 20–22). Systemic availability leading to immune involvement could then be attributed to shunts present in the area of exposure and interfacial defects at sites of lipid phase separation, demonstrated to be an alternative, albeit limited pathway for ion transport through mammalian skin (23, 24). The limited and extremely slow diffusion across the SC in the case of nickel ($Kps = 10^{-6} – 10^{-4}$) does not adequately explain the rapid onset of skin reactions in nickel-sensitive individuals upon contact with nickel compounds. Similarly, in the same *in vitro* stripping experiment where the depth profile of nickel is characterized after application at 5% as the chloride (12), the 30%-plus of the dose remaining unaccounted for may also be explained in part by shunt diffusion, which evades strip analysis.

Since in our experiment unadsorbed permeant and SC were removed after relatively short residence times, and stripping involved areas ($6.45 cm^2$) which considerably exceeded those of treatment ($2.83 cm^2$), incomplete recovery of the dose applied may not be attributed to lateral diffusion, which potentially could result in migration beyond the stripped area (25, 26). Significant lateral diffusion by nickel within this timeframe is further unlikely due to its affinity for SC keratin. Also, since stripping of the SC does not result in the quantitative removal of hair follicles (27), an indeterminate part of the dose immobilized in the follicular epithelium will elude detection.

Differences in penetration depth and recovery values between our results and those described in Fullerton et al.'s *in vitro* experiments with full-thickness human skin (12, 28) invite comment, particularly since *in vitro* the nickel found in the epidermis reached 11%. Fullerton et al. observed that nickel uptake in the epidermis directly correlates with and is dependent on the initial concentration applied. Our surface "disappearance" values became measurable at higher concentrations too. Nickel application concentration by Fullerton et al. was 5%; the highest in our experiment was 1%. The gender of the skin sources and anatomical sites of skin exposed in the two experiments were different; if, as we anticipate, shunt diffusion is an important pathway in the penetration process (30% plus of the dose applied by Fullerton et al. remained unaccounted for), then the shunt density in skin involved may also contribute to the differences. Finally, over the 96-h duration of the (occluded) *in vitro* experiment by Fullerton et al., a substantial part of the skin barrier function would be expected to be lost, facilitating diffusion.

Nickel adsorption profiles in the SC for the four salts and variable experimental conditions only differ quantitatively. The increasing gradients across the SC and their converging decline towards the limit of detection, regardless of exposure dose, time or site applied, are consistent with earlier observations that nickel has considerable affinity for SC proteins, imparting substantivity for the superficial layers of the epidermis, resulting in depot formation (2, 9, 12, 29, 30)

Solubility of the 4 salts in n-octanol ranges over 4 orders of magnitude (Table II). The observed effect of counter ion on the diffusivity of nickel salts may at least in part cause such a significant difference in their polarity (lipophilicity), reflecting covalency and ionic interactions. Nickel nitrate, the most lipophilic ion pair, represents a notable departure from the monotonic concentration curves of the other salts, as it progresses at a constant concentration into the deeper SC (Fig. 4). Also the AUC for that salt, indicating the amount retained in the SC after surface decontamination is significantly higher (AUC = $26.1 \pm 1.2$; $p < 0.0005$) than are totals for the other salts applied at comparable Ni (II) concentrations (AUC = $3.3 \pm 0.6$ to $18.5 \pm 4.1$). A reasonable explanation would be the choice of an alternate diffusion pathway and deposition by the nitrate, i.e. via the intercellular lipid domains. The salt with the most covalency may thus achieve greatest diffusivity through the SC barrier over time.

Percent total recovery of nickel as nitrate from strip analysis is directionally less from arm application ($72.0 \pm 3.0$) than recovery from back skin ($87.5 \pm 11.8$) at the same concentration (Table I), taken as an indication of a regional difference in diffusivity under otherwise identical conditions. This is also apparent as AUC for the chloride (Fig. 3), where the difference between nickel retained in arm and back SC is significant ($p < 0.05$). This is in accordance with observations made by others of more facile diffusion through arm skin than the back, with concomitant differences in irritative response between the two sites (3, 6, 31). Such regional differences in skin permeability, exhibited especially for hydrophilic permeants, are apparently due not so much to differences in thickness of the SC as to the lipid weight percentage and differences in the compositional profile of SC lipids over different skin sites (32, 33). This may also serve to explain the frequency of false-negative reactions observed in diagnostic patch testing routinely performed on the back using the sulfate salt (34–37).

The hierarchy in polarity measured for the nickel salts corresponds to the degree of irritancy (and thus diffusivity) in the skin: nitrate > chloride > acetate > sulfate, also observed earlier by others (3, 6, 7). Nickel thus appears to transit as an ion-pair complex, closely associated with its counter ion, constituting a steric factor which impacts on diffusivity, further amplified by the ion pair hydration spheres. Transfer of charged ions as ion pair has been stipulated as one mechanism of permeation, and the effect of counter ion on permeant size, polarizability and polarity, ultimately expressed in skin diffusivity, has been demonstrated for ionic compounds (38, 39). As the water content of the SC progressively increases in the deeper layers, degree of ion (complex) hydration increases further, to become fully hydrated (i.e. primary and secondary hydration spheres) towards the deeper regions of the membrane. The resulting increased steric hindrance also leads to a decrease in ion mobility.

Our stripping experiments, at least in part, conform to that sequence in biological activity, assuming that the AUC recorded for sulfate, acetate and nitrate is an indication of their diffusivity and eventual penetration to the viable epidermis. Those values differ significantly among each other (Table II), depending on their polarity. The chloride falls

outside that order, however, evidently subject to other factors determining its diffusivity, which we cannot explain at this stage.

*Clinical observations.* After 2 years of repeated and prolonged exposure to relatively high concentrations of nickel salts (up to 1%) and to micronized metallic nickel under occlusion (to be reported elsewhere), none of the volunteers developed clinical signs of nickel sensitivity. Such lack of sensitization was not confirmed by patch testing, however.

The process of multiple stripping on the same subject at sites in the same general area of the skin caused different degrees of irritation, an expression of the intra-individual variability generally noted in skin testing. Areas of application and stripping progressing from the wrist towards the antecubital fossa showed a gradual increase in erythema formation after the same number of strips – an indication of a gradual change in SC thickness on the arm, as observed earlier by others (40, 41). This points to the extent of intra-individual variability in the barrier properties of the skin, both spacially and temporally, of any individual. Results obtained by SC stripping thus remain subjective and are prejudicial in the endeavor to arrive at general conclusions based on the relatively limited scale of our serial investigations. This variability accounts at least in part for the margins of error that characterize experimental replicates.

In conclusion, as nickel appears to penetrate the SC to a limited extent only, the bricks-and-mortar array functions as an effective barrier for strong electrophiles coming into contact with the skin. The remarkably long lag times for *in vitro* diffusion of nickel can be explained by such an effective barrier function. While amounts of the allergen that do reach the viable structures of the epidermis appear to depend on limited shunt diffusion, they are nevertheless sufficient to elicit allergic reactions.

Penetration of the SC by nickel salts is limited and closely related to the counter ion. Most of the dose remains on the surface or is retained in the superficial layers of the SC. Nickel salts appear to follow alternative, competing routes of penetration: the slow transcellular and intercellular pathways as well as the rapid transit through the skin shunts. Considering that nickel is a premier contact allergen in industrialized society, this alternative, relatively rapid mode of penetration may explain facile elicitation of the widespread immune reactions observed.

With the example of the nickel ion, the present investigation points to the complexities involved in skin penetration by charged molecules, transition metal ions in particular. Polarity, which has a bearing on the route of penetration; ion pairing, the bulk of which includes the hydration sphere; electrophilicity, or, in the case of essential elements, effects due to homeostatic control; all appear as factors defining the diffusion process. Nickel as permeant is also evidence of the characteristics of the skin as a barrier, a reservoir, and a filter. ICP-AES, with a sensitivity of 20 ppb, made these observations possible. Such *in vivo* data aid in the interpretation of previous *in vitro* studies, and may provide insight for possible interventions towards decreasing nickel allergy.

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