Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy

Volume 16 Article 4

2011

Nickel Dermal Bioavailability in Pig Skin Increased by a Chemical Mixture: Role of Gender

Mohamed S. Abdel-Rahman Ph.D.
University of Medicine and Dentistry of New Jersey, abdelrms@umdnj.edu

Rita M. Turkall Ph.D.
University of Medicine and Dentistry of New Jersey, turkalrm@umdnj.edu

Nickel Dermal Bioavailability in Pig Skin Increased by a Chemical Mixture: Role of Gender

Cover Page Footnote
§ Corresponding Author: Mohamed S. Abdel-Rahman, Ph.D., F.C.P., B.C.F.E., Pharmacology and Physiology Department, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, Newark, New Jersey, USA, 07103-2714; Telephone: 973-979-3146; Email: abdelrms@umdnj.

Part II: Heavy Metals

Chapter 3

NICKEL DERMAL BIOAVAILABILITY IN PIG SKIN INCREASED BY A CHEMICAL MIXTURE: ROLE OF GENDER

Nickel Dermal Bioavailability Increased by a Chemical Mixture

M.S. Abdel-Rahman[1,§] and R.M. Turkall[1,2]

[1]University of Medicine and Dentistry of New Jersey, Pharmacology and Physiology Department, New Jersey Medical School and [2]Clinical Laboratory Sciences Department, School of Health Related Professions, Newark, NJ

ABSTRACT

Exposure to chemical mixtures is more common than exposure to a single chemical. Skin is the largest tissue in the human body and is an important route of exposure to chemical mixtures. The aim of this study was to assess the effect of toluene, trichloroethylene (TCE) and phenol on the dermal bioavailability of nickel. All four compounds are prevalent in the environment, at industrial facilities, and at hazardous waste sites. An *in vitro* approach was employed which utilized radiotracer methodology and a modified Teflon flow-through diffusion cell system to measure the amount of chemical which penetrated through or became bound to dermatomed male or female pig skin. In males, there was almost a 2-fold increase in the total cumulative percentage of radioactivity in the receptor fluid after treatment with the mixture compared to nickel alone. In females, significantly more radioactivity (2-fold) penetrated into receptor fluid when skin was treated with the chemical mixture of nickel versus nickel alone. The chemical mixture produced a significant increase in the total penetration and the amount of nickel that became bound to skin relative to nickel alone in both sexes. Also, more radioactivity remained loosely adsorbed to skin and could be easily washed off of

§ Corresponding Author: Mohamed S. Abdel-Rahman, Ph.D., F.C.P., B.C.F.E., Pharmacology and Physiology Department, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, Newark, New Jersey, USA, 07103-2714; Tel: 973-979-3146; Email: abdelrms@umdnj.edu

the skin surface when nickel was applied alone rather than in combination to male or female skin. However, the total penetration and the radioactivity in the skin matrix were significantly higher in females than in males treated with the nickel mixture. This study revealed that the bioavailability of nickel to skin is significantly higher when administered in the chemical mixture compared to nickel alone. Furthermore, females are at greater risk than males from dermal exposure to the nickel mixture.

Keywords: nickel bioavailability, dermal exposure, mixture effects, gender differences

1. INTRODUCTION

Occupational and environmental exposures to chemicals occur more often to mixtures rather than to a single compound (Ogata *et al.*, 1993) Because of its extensive surface area, skin is a major route of exposure to chemical mixtures. When dermal exposure occurs simultaneously to two or more chemicals, the bioavailability of the mixture can be altered relative to that predicted by the separate components of the mature. In order to accurately assess the human health risks from dermal exposure to chemical mixtures, data on the bioavailability of the mixtures is needed. The purpose of this research was to utilize an *in vitro* approach consisting of Teflon flow-through diffusion cell methodology and radiotracer techniques to determine the affects of a mixture of toluene, TCE and phenol on the *in vitro* dermal bioavailability of nickel. Each of the chemicals in the mixture is prevalent in hazardous waste sites as well as at industrial facilities, and in the environment. Several million workers worldwide are exposed to nickel resulting in excess incidences of cancer of the nasal cavity and the lungs. However, the most frequent health effect from chronic exposure to nickel in humans is allergic contact dermatitis (ATSDR, 1993a). Although the best *in vitro* model for human risk assessment is human skin, the source of human skin is limited and, for comparison purposes, it is difficult to control the gender, race, anatomical site, age and condition of the donor skin. Pig skin, having many morphological and functional characteristics similar to human skin, is considered one of the best animal models for penetration studies and was used in the investigation (Meyer *et al.*, 1978).

2. MATERIALS AND METHODS

2.1 Chemicals

^{63}Nickel chloride, having a specific activity of 12.63 mCi/mg and radiochemical purity of 99.9%, was obtained from E.l. Dupont de Nemours and Co., Inc., New England Nuclear (NEN) Research Products, Boston, MA.

2.2 Animal Model

Whole pig skin was obtained from the costo-abdominal areas of euthanized (40-60 lb) Yorkshire pigs (Cook College Farm, Rutgers University, New Brunswick, NJ). The pig has been widely accepted as an animal model for studying human percutaneous absorption of a large variety of chemicals under various experimental conditions (Bartek *et al*., 1972; Reifenrath and Hawkins, 1986) because of the well documented histological (Monteiro-Riviere and Stromberg, 1985), physiological, biochemical, and pharmacological similarities between pig skin and human skin (Qiao and Riviere, 2000). Skin was transported to the laboratory in ice-cold HEPES buffered (25 mM) Hank's balanced salt solution (HHBSS), pH 7.4, containing gentamycin sulfate (50 mg/l) (Collier et al., 1989) after which it was immediately prepared for diffusion cells according to Bronaugh and Stewart (1985).

2.3 In Vitro Dermal Penetration Studies

Excised skin was cut to a thickness of 200 υm with a dermatome (Padgett Electro-Dermatome model B, Padgett Instruments Inc., Kansas City, MO) and mounted into Teflon flow-through diffusion cells (Crown Bio Scientific Inc., Clinton, NJ). The exposed skin surface area was 0.64 cm^2 and was maintained at a temperature of 32°C. The dermal side of each skin sample was bathed with HHBSS receptor fluid containing 10% fetal bovine serum (Sigma Chemical Co., St Louis, MO) at a flow rate of 5 ml/h by a multichannel peristaltic cassette pump (Manostat, NY) and aerated continuously with 99.9% oxygen (Collier *et al.*1989). ^{63}Nickel chloride was administered either individually or in the mixture to the stratum corneum surface of the epidermis in a total volume of 10 υL. The chemical dose of nickel chloride was 100 ng/cm^2 containing 0.92 υCi of radioisotope. The chemical doses of the non-labeled chemicals were 5.8 mg TCE, 3.4 mg toluene, and 6.8 υg phenol/cm^2. Perfusate was collected at 15 minute intervals up to 1 h, at 1.5 and 2 h, then at 2 h intervals up to 16 h postdosing.

At the conclusion of the 16 h study, a gentle stream of air was allowed to flow over the skin surface for 1 h. Any of the radioactive compound which volatilized

from the surface could be extracted with hydrochloric acid from charcoal tubes (SKC Inc., Eighty-Four, PA) attached to the upper chambers of the diffusion cells. Unabsorbed chemical was washed off of the skin surface with 1% aqueous soap solution followed by distilled water. Skin samples were completely solubilized in Solvable (NEN) to determine the binding capacity of the skin. Radioactivity was counted in Formula 989 liquid scintillation cocktail (NEN) by a Beckman LS-7500 spectrometer. Sample quench was corrected using the H-ratio method.

2.4 Statistical Analysis

All data were reported as the mean $\pm$ standard error of the mean (SEM). Statistical differences between treatment groups were determined by Student's independent t-test. The level of significance was $p < 0.05$.

3. RESULTS AND DISCUSSION

The cumulative penetration of nickel was used to describe the total amount as percent of initial nickel dose which permeated skin and appeared in receptor fluid within a designated time interval. In males, the total cumulative percentage of radioactivity in receptor fluid at 16 h was increased almost 2-fold in the mixture compared to nickel alone (Table 1).

Table 1. Cumulative penetration of ^{63} nickel alone or in a chemical mixture through male pig skin into receptor fluid

Time (h)	Nickel Alone	Nickel Mixture [a]
0-4	0.1 ± 0.0[b]	0.3 ±0.1[c]
0-8	0.2 ± 0.0	0.5 ± 0.1[c]
0-12	0.3 ± 0.1	0.6 ± 0.1[c]
0-16	0.4± 0.1	0.7 ± 0.1

[a] Mixture consists of ^{63}nickel, toluene, TCE and phenol.

[b] Values (mean ± S.E.M.) represent the percentage of the initial dose collected in the receptor fluid at the indicated time, from 12-17 replicates per treatment *in vitro*.

[c] Significantly different from nickel alone, t-test ($p < 0.05$).

In females, there was a significant increase in the dermal bioavailability of nickel when exposure occurred in the presence of toluene, TCE and phenol (Table 2). This was supported by the significantly higher cumulative percentage of nickel in a mixture penetrating skin into receptor fluid throughout the study. At the end of the 16 h study, the total cumulative percentage of the nickel mixture was 2-fold higher than nickel alone.

A summary of the amount of nickel-derived radioactivity that penetrated pig skin into receptor fluid as well as the binding capacity of nickel to pig skin is shown in Tables 3 and 4. In males treated with the mixture, significantly more

Table 2. Cumulative penetration of ^{63} nickel alone or in a chemical mixture through female pig skin into receptor fluid

Time (h)	Nickel Alone	Nickel Mixture[a]
0-4	0.1 ± 0.0.0[b]	0.3 ±0.1[c]
0-8	0.2 ± 0.0	0.6 ± 0.1[c]
0-12	0.3 ± 0.0	0.8 ± 0.1[c]
0-16	0.5 ± 0.1	1.0 ± 0.1[c]

[a] Mixture consists of ^{63}nickel, toluene, TCE and phenol.
[b] Values (mean ± S.E.M.) represent the percentage of the initial dose collected in the receptor fluid at the indicated time, from 12-16 replicates per treatment *in vitro*.
[c] Significantly different from nickel alone, t-test ($p < 0.05$).

radioactivity remained bound to skin (68.9% of the initial dose) compared to nickel alone (57.6%) (Table 3). The total amount of absorbed nickel that is available for distribution to the body (total penetration) is the sum of the total dose in the receptor fluid and bound to skin. Total penetration of nickel was significantly increased in males when nickel was applied as a mixture to skin (69.5%) versus nickel atone (57.9%). At the same time, significantly less radioactivity was found in the skin wash of the mixture (26.5%) than nickel alone (34.3%).

Table 3. Summary of the penetration of ^{6S} nickel alone or in a chemical mixture through male pig skin

	Nickel Alone	Nickel Mixture[a]
Receptor Fluid	0.4 ± 0.1 [b]	0.7 ±0.1[c]
Skin Digest	57.6 ± 2.2	68.9 ± 3.6[c]
Total Penetration	57.9 ± 2.2	69.5 ± 3.6[c]
Skin Wash	34.3 ± 2.0	26.5 ± 3.4[c]

[a] Mixture consists of ^{63}nickel, toluene, TCE and phenol.
[b] Values (mean ± S.E.M.) represent the percentage of the initial dose recovered at the end of the 16 h study (n = 12-17 replicates per treatment) *in vitro*. Total penetration is the sum of radioactivity in the receptor fluid and bound to skin. There was no volatilization from the skin surface.
[c] Significantly different from nickel alone, t-test ($p < 0.05$).

Similar results were observed in females (Table 4). The amount of radioactivity bound to skin after treatment with the nickel mixture (79.3% of the initial dose) and the total penetration (80%) were significantly higher than nickel by itself (57.7% and 58.1%, respectively). Females also showed a significant decrease in radioactivity when skin was washed with soap and water after treatment with the mixture (26.3%) versus nickel alone (41.5%).

Table 4. Summary of the penetration of ^{63}nickel alone or in a chemical mixture through female pig skin

	Nickel Alone	**Nickel Mixture[a]**
Receptor Fluid	0.5 ± 0.1[b]	1.0 ±0.1[c]
Skin Digest	57.7 ± 2.3	79.3 ± 2.9[c]
Total Penetration	58.1 ± 2.3	80.0 ±2.8[c]
Skin Wash	41.5 ±2.2	26.3 ± 2.4[c]

[a] Mixture consists of ^{63}nickel, toluene, TCE and phenol.
[b] Values (mean ± S.E.M.) represent the percentage of the initial dose recovered at the end of the 16 h study (n = 12-16 replicates per treatment) *in vitro*. Total penetration is the sum of radioactivity in the receptor fluid and bound to skin. There was no volatilization from the skin surface.
[c] Significantly different from nickel alone, t-test ($p < 0.05$).

When a comparison was made between males and females to assess the penetration of nickel from the mixture, the total penetration of radioactivity and the amount bound to the skin matrix were significantly higher in females than in males (Table 5).

Table 5. Comparison of the penetration of ^{63}nickel in a chemical mixture through male and female pig skin [a]

	Male	**Female**
Receptor Fluid	0.7 ± 0.1[b]	1.0 ± 0.1
Skin Digest	68.9 ± 3.6	79.3 ± 2.9[c]
Total Penetration	69.5 ± 3.6	80.0 ± 2.6[c]
Skin Wash	26.5 ± 3.4	26.3 ± 2.3

[a] Mixture consists of ^{63}nickel, toluene, TCE and phenol.
[b] Values (mean ± S.E.M.) represent the percentage of the initial dose collected in the receptor fluid at the indicated time, from 12-17 or 12-16 replicates per treatment *in vitro*, for males and females, respectively.
[c] Significantly different from male, t-test ($p < 0.05$).

4. CONCLUSIONS

This study demonstrates that ionic nickel binds to and can penetrate pig skin, as has been shown previously in human skin (Larese *et al*., 2007). These results support the dermal bioavailability of nickel and are consistent with the metal's ability to produce allergic contact dermatitis, a serious health hazard resulting from the exposure to nickel in the environment due to pollution, in the workplace, and during daily contact with items such as coins, jewelry, and stainless steel products (Gazel, *et al.*, 2008). Moreover, the effect of the phenol-toluene-TCE mixture was to significantly increase the dermal penetration as well as skin binding of nickel in both male and female skin. This finding supports increased

nickel bioavailability resulting from dermal exposure to the mixture and, thus, increased health risk particularly in females whose results were significantly higher than males.

The protein denaturing action of phenol together with the defatting action of toluene and TCE (Roberts *et al.*, 1977, ATSDR, 1992, ATSDR, 1993b) on skin may be contributing factors to increased penetration of nickel in the mixture. Alterations in skin integrity induced by the chemical mixture in this study are consistent with increased penetration of nickel in human skin that had been physically abraided (Larese-Filon, F, *et al*., 2009). These findings underscore the need for workers exposed to nickel under similar mixture conditions to use protective clothing, such as gloves.

The gender differences revealed in this study are the first to be reported for nickel. The mechanisms by which these differences arise are not completely understood. However, a previous *in vitro* study in our laboratory (McCormick and Abdel-Rahman, 1991) showed similar results, with TCE dermal penetration greater in the skin of female than male rats. The maintenance of skin moisture and thickness and enhancement of keratinocyte proliferation by estrogen (Kanda and Watanabe, 2005) may also play a role in greater nickel penetration and binding in females. Further studies are required to explore these possibilities.

5. REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry) 1992. Toxicological Profile for Toluene, p. 49. U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.

ATSDR (Agency for Toxic Substances and Disease Registry) 1993a. Toxicological Profile for Nickel, p. 46. U.S. Department of Health and Human Services, Pubic Health Service, Atlanta, Georgia.

ATSDR (Agency for Toxic Substances and Disease Registry) 1993b. Toxicological Profile for Trichloroethylene, p. 39. U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.

Bartek, M.J., LaBudde, J.A. and Maibach, H.I. 1972. Skin permeability *in vivo*: Comparison in rat, rabbit, pig, and man. J. Invest. Dermatol. 58, 114-123.

Bronaugh, R.L. and Stewart, R.F. 1985. Methods for *in vitro* percutaneous absorption studies. IV. The flow-through diffusion cell. J. Pharm. Sci. 24, 64-67.

Collier, S.W., Sheikh, N.M., Sakr, A., Lichtin, J.L , Stewart, R.F., and Bronaugh, R.L. 1989. Maintenance of skin viability during *in vitro* percutaneous absorption/metabolism studies. Toxicol. Appl. Pharmacol. 99, 522-533.

Gazel, A., Rosdy, M., Tornier, C., De Fraissinette, A and Blumenberg, M. 2008. Transcriptional profiling defines the effects of nickel in human epidermal keratinocytes. J. Cell Physiol. 217, 686-692.

Kanda, N. and Watanabe, S. 2005. Regulatory roles of sex hormones in cutaneous biology and immunology. J. Dermatol. Sci. 38, 1-7.

Larese, F., Gianpietro, A., Venier, M., Maina, G. and Renzi, N. 2007. *In vitro* percutaneous absorption of metal compounds. Toxicol. Letters 70, 49-56.

Larese-Filon, F., D'Agostin, F., Grosera, M., Adami, G., Bovenzi, M. and Maina, G. 2009. *In vitro* absorption of metal powders through intact and damaged human skin. Toxicol. In Vitro 23, 574-579.

McCormick, K. and Abdel-Rahman, M.S. 1991. The role of testosterone in trichloroethylene penetration *in vitro.* Environ. Research 54, 82-92.

Meyer, W., Schwarz, R. and Neurand, K. 1978. The skin of domestic mammals as a model for the human skin with special reference to the domestic pig. Curr. Probl. Dermatol. 7, 39-52.

Monteiro-Riviere, N. and Stromberg, M. 1985. Ultrastructure of the integument of the domestic pig (Sus scrofa) from one through fourteen weeks of age. Anat. Histol. Embryol. 14, 97-115.

Ogata, M., Fiserova-Bergerova, V. and Droz, P.O. 1993. Biological monitoring VII: Occupational exposures to mixtures of industrial chemicals, Appl. Occup. Environ. Hyg. 8, 609-617.

Qiao, G. and Riviere, J.E. 2000. Dermal absorption and tissue distribution of 3,3', 4,4'-tetrachlorobiphenyl (TCB) in an *ex-vivo* pig model: Assessing the impact of dermal exposure variables. Int. J. Occup. Environ. Health, 6, 127-137.

Reifenrath, W. and Hawkins, G. 1986. The weanling Yorkshire pig as an animal model for measuring percutaneous penetration. In: Swine in Biomedical Research, pp. 673-680. (Tumbelson, M.E., Ed.). New York, Plenum.

Roberts, M.J.S,, Anderson, R.A. and Swarbrick, J. 1977. Permeability of human epidermis to phenolic compounds. J. Pham. Pharmacol. 29, 67-83.