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In Vitro Skin Absorption of ^{14}C-Cyanuric Acid in a Simulated Swimming Pool

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Cyanuric acid (1,3,5-triazine-2,4,6(1H,3H,5H)-trione) is commonly used in public and private swimming pools to decrease the photodegradation of the chlorine used for disinfection purposes. By stabilizing available chlorine levels, cyanuric acid enhances the efficacy of sanitation while decreasing costs by lowering the frequency of chlorine treatments (Canelli, 1974); Downes et al., 1982). While CYA is not acutely toxic (oral LD50 in rats > 5 g/kg bw), it has been shown to cause lesions of the kidney and bladder in the rat following prolonged exposure through drinking water (EPA 1988). The nature of swimming is such, that the potential for exposure to pool chemicals through swallowing water and/or dermal absorption is quite high. High performance athletes in training are known to spend up to 4 hours/day in the pool for 300 days/year, and are estimated to swallow up to 60 mL/hr of pool water (Datta, 1979).

Wester et al. (1987) have recently reported that swimmers and bathers may acquire toxicologically significant residues of chemical contaminants via skin absorption. Inokuchi et al. (1978) reported that little or no radiolabelled CYA penetrated skin of rats when the CYA was applied to the animals using the occluded patch technique, however, this procedure does not reflect the human exposure situation. Allen et al. (1982) reported cumulative recovery of CYA in the urine of swimmers, 20 hr after swimming, averaging 9.8 mg, however, it is not possible to determine what proportion, if any, of the CYA came from dermal absorption. As part of this same study, an attempt was made to measure dermal absorption of cyanuric acid in 5 human volunteers who soaked in a pool for 2 hours. The data available showed such a wide variation (100 fold) in excretion of CYA between subjects that no accurate estimation of dermal absorption was possible.

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We report here the results of an investigation designed to quantify dermal penetration of CYA under simulated swimming pool exposure conditions, in an in vitro test system. We have previously reported an automated in vitro dermal absorption (AIDA) method for investigating skin absorption of pesticides (Moody and Martineau, 1990). and have modified the method of applying the donor (treatment) solution in the present case to permit an 'infinite, circulating' reservoir of CYA to model the exposure conditions existing in the swimming pool.

Skin from humans and several animal species was tested to determine the relationship between dermal absorption in animal models to humans. A tissue cultured human-derived skin (Testskin®, Organogenesis Inc.) was also evaluated as an alternative to actual human skin.

MATERIALS AND METHODS

^{14}C-CYA (SA 10.1 mCi/mM, radiochemical purity > 95%) was kindly supplied by Monsanto Co., St. Louis, Mo. ^{14}C-DEET (diethyl-m-toluamide) (SA 4.4 mCi/mM, radiochemical purity > 95%) was obtained from Dupont New England Nuclear, Lachine, PQ, and was used as a positive control. The effect of the receiver solution (Ringer's or Hank's) on skin absorption of CYA was tested. Preparation of Ringer's receiver solution was as described previously (Moody and Martineau, 1990). Hank's receiver solution contained 1 vial of Hank's balanced salts with NaCl reduced to 7 g/L (Sigma Chemical Co., St. Louis, Mo), 5.96 g Hepes buffer, 0.35 g $NaHCO_3$, 0.05 g Gentamycin sulfate and 40 g bovine serum albumen per litre of distilled water. Both receiver solutions were adjusted to pH 7.4 with 1 N NaOH and then sterilized by passing through a 0.2 μM filter.

Skin specimens were obtained from the shaved mid-dorsal region of Sprague Dawley rats and from the back of hairless guinea pigs immediately post-euthanasia with barbiturate. Human abdominal skin of a female Caucasian (43 yr) was obtained viable from a local hospital and was mounted in the AIDA chamber within 1 hr of surgery. All skins except Testskin® (Organogenesis Inc., Cambridge, Ma) were dermatomed as described previously (Moody and Martineau, 1990).

The method of skin absorption analysis followed that reported previously (Moody and Martineau, 1990; Moody and Ritter, 1991) except that the donor solution was delivered at a rate of 5 mL/min over the skin surface with a peristaltic pump (Watson-Marlow, Cornwall, U.K.). The donor solution simulated the swimming pool water (see Fig. 1) and consisted of 100 mL distilled water containing either 1 μCi or 100 μCi of ^{14}C-CYA, unlabelled

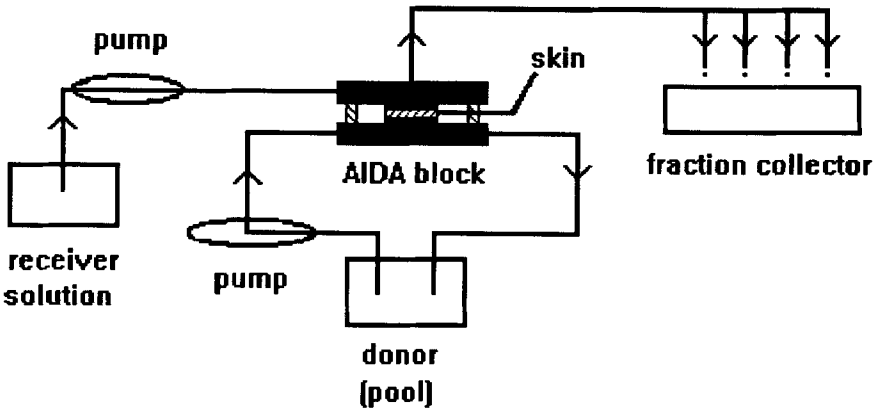

Figure 1. Flow diagram of the AIDA procedure for monitoring skin absorption of chemicals in a model swimming pool. (for details of the aluminum AIDA cell chamber (block) see Moody and Martineau, 1990).

CYA at 55 ppm, and chlorine at 1.5 ppm as specified in Tables 1 and 2. In our initial studies, 1 μCi of ^{14}C-CYA was added to the pool. After the data demonstrated only minimal dermal absorption, 100 μCi of ^{14}C-CYA were used in order to ensure detection of ^{14}C-activity if less permeable skin (e.g. human) was used (see footnotes to Tables 1 and 2). The concentrations of unlabelled CYA and chlorine were selected as being representative of standard pool conditions (Wojtowicz et al., 1984). Donor solution free chlorine concentration was determined with a pool test kit (Aquality Inc., Chatsworth, Ca). Donor solution pH was adjusted to 7.4 using $NaHCO_3$ since bicarbonate is in common use as a pool water buffer. ^{3}H-H_2O (1 μCi) was also added to the donor pool to ensure skin sample barrier integrity (Bronaugh et al., 1986). In all cases, total skin absorption of ^{3}H-H_2O by 24 hr post-application was < 0.01%. The donor solution was contained in a 125 mL Erlenmeyer flask and was pumped in recirculating fashion over the outer epidermal side of the skin (Fig. 1). The receiver solution simulating blood, was pumped at 40 μL/min over the inner dermal side of the skin. The AIDA block and donor (pool) solution were maintained at 37°C. Receiver samples were harvested in a fraction collector (Fig. 1) every 2 hr for a 24 hr period and analyzed by liquid scintillation counting as reported previously (Moody and Martineau, 1990). Pharmacokinetic analysis of the data was performed by electronic spreadsheet as detailed previously (Moody and Ritter, 1991). Analysis of methanol skin washes and tissue digests of the skin at 24 hr post-application was conducted as detailed previously.

RESULTS AND DISCUSSION

Table 1 demonstrates that only very low percent recovery of ^{14}C-CYA was obtained in the treated skin specimens in the methanol skin washes and skin tissue digests (bound-residues) harvested at the end of each AIDA analysis (at 24 hr). No detectable ^{14}C-activity was observed in the human and Testskin® samples. In contrast 25% of the applied dose was obtained in the wash + digest samples when ^{14}C-CYA was applied as a finite dose (1 μCi/10 μL acetone) to rat skin following the procedure of Moody and Ritter (1991).

Table 2 summarizes the dermal absorption data. In all cases the % dermal absorption, the cumulative amount of CYA absorbed and the maximum rate of permeation ranged from non-detectable to minimal. In probably the most pertinent case, where human skin was tested with a pool composition including unlabelled CYA and chlorine, only 0.06 μg/cm^2 total cumulative absorption was detected over the 24 hr exposure period. Employing the value of 1.83 M^2 used by Guy and Maibach (1984) for the total body surface area of a 70 kg human, 0.06 μg/cm^2 implies a total body exposure of 1.1 mg for a 24 hr exposure period. Assuming a 'worse-case' maximum exposure time of 5 hr daily, the data suggests that 0.2 mg/day would be absorbed through a swimmer's skin. Given that for a standard water CYA concentration of 55 ppm (mg/L) that 0.2 mg would be contained by 3.6 mL of pool water, it seems reasonable to predict that exposure <u>via</u> the oral route could easily supersede that of the dermal, especially when considering the report of Datta (1979) that swimmers can swallow up to 60/mL/hr of pool water.

When cross-comparing the data in Table 2, it is apparent that the presence of unlabelled cyanurate (CC) in the "pool" markedly affected the calculations when expressed as μg of CYA (last 2 columns Table 2) since the total μg for both the ^{14}C-labelled and unlabelled CYA was calculated. While the % skin permeation rates in the first two studies reported in Table 2, Rat (Ring) and Rat (Hank), were similar (0.003 and 0.004), the Rp values were quite different (0.00 and 0.06 μg/cm^2/h), due to the much larger total dose of CYA in the latter study.

It is interesting to compare the data obtained where 1 μCi of ^{14}C-DEET (87 ug/100 mL pool) was added to the pool in place of 1 μCi of ^{14}C-CYA (11.2 ug/100 mL pool). DEET is rapidly absorbed through the skin (Moody and Martineau, 1990) and was used here as a positive control for dermal absorption. Approximately 23 fold (0.07/0.003) greater % permeation of DEET was obtained in rat skin in comparison to the % permeation obtained for CYA in the

comparable CYA study (Rat (Ring)) in Table 2. This is consistent with the observation that 36% permeation of DEET in rat skin was observed in an AIDA study conducted with Hank's receiver in a finite dose study (unpublished data), in that this was much greater than the 7% permeation of CYA obtained in the finite dose study with rat skin and Hank's receiver here (Table 2) (a DEET/CYA % permeation ratio of 5). Hence DEET permeates more readily through rat skin than CYA in both finite and infinite type dose applications. Albeit the **finite** dose data suggests that predictions of the relative permeability of environmental contaminants could be made for swimmers and others exposed to an infinite dose, the disagreement of the permeation ratios (23 (infinite) versus 5 (finite)) implies that such predictions would not be accurate. Hence we conclude simply, that in order to best predict the % skin permeation of a chemical present in infinite dose, it is necessary to conduct a flow-through type infinite dose procedure as has been developed in our laboratory using the AIDA procedure.

Table 1. In vitro % recovery data from skin following exposure to ^{14}C-cyanurate in a model swimming pool.

Species	R&H[d]	Pool Parameters[e]	Wash(W)[a] (%)	Digest(D)[b] (%)	W+D[c] (%)
Rat	Ring	DW,*	0.23 ± 0.12	0.05 ± 0.02	0.28 ± 0.14
Rat	Hank	DW,CC,CL	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Rat	Ring	DW,**DEET**,*	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.02
Guinea Pig	Ring	DW,*	0.00 ± 0.00	0.17 ± 0.04	0.17 ± 0.04
Guinea Pig	Hank	DW,CC,CL	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.02
Human	Ring	DW,CC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Human	Hank	DW,CC,CL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testskin^{R}	Ring	DW,CC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Finite Dose Application (see text for explanation)					
Rat	Hank	Acetone	14.9 ± 3.66	10.5 ± 2.28	25.4 ± 3.82

Values are means ± SD (n = 4) with exception of the human study with Hank's receiver solution (n = 3). Wash[a] is % recovered in methanol washes of skin at 24 h; Digest[b] is % recovered in skin digest; W+D[c] is % total recovered in methanol wash and skin digest. R&H[d] specifies the receiver solution (Ringer's or Hank's) used in each study. Pool Parameters[e] specify the parameters used to create the pool donor solution including; DW (distilled water (100 mL)), CC (unlabelled cyanurate (55 ppm)), CL (chlorine (1.5 ppm) as well as the ^{14}C-cyanurate and $^{3}H_2O$ radiolabels that were present in all cases. An * indicates use of 1 μCi of ^{14}C-labelled CYA or DEET (otherwise 100 μCi ^{14}C-CYA were applied). In the finite dose study 0.1 μCi of ^{14}C-DEET was applied. Note that **DEET** indicated above in the pool parameters indicates a study with rat skin where ^{14}C-DEET was used in place of ^{14}C-cyanurate as detailed in the text.

Table 2. In vitro pharmacokinetic data for different species of animal skin following exposure to ^{14}C-cyanurate in a model swimming pool.

Species	R&H[e]	Pool Parameters[f]	Th[a] (mm)	Per[b] (%)	Cum[c] ($\mu g/cm^2$)	Rp[d] ($\mu g/cm^2/h$)
Rat	Ring	DW,*	0.50 ± 0.01	0.003 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Rat	Hank	DW,CC,CL	0.64 ± 0.05	0.004 ± 0.00	1.12 ± 0.97	0.06 ± 0.05
Rat	Ring	DW,**DEET**,*	0.50 ± 0.00	0.070 ± 0.08	0.30 ± 0.37	0.07 ± 0.10
Guinea Pig	Ring	DW,*	0.31 ± 0.01	0.076 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
Guinea Pig	Hank	DW,CC,CL	0.50 ± 0.01	0.001 ± 0.00	0.20 ± 0.34	0.01 ± 0.02
Human	Ring	DW,CC	0.50 ± 0.01	0.000 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Human	Hank	DW,CC,CL	0.51 ± 0.01	0.000 ± 0.00	0.06 ± 0.04	0.00 ± 0.00
Testskin^R	Ring	DW,CC	0.30 ± (**)	0.000 ± 0.00	0.02 ± 0.03	0.00 ± 0.00
Finite Dose Application (see text for explanation)						
Rat	Hank	Acetone	0.50 ± 0.01	7.07 ± 2.84	0.35 ± 0.14	0.05 ± 0.02

Values are means ± SD (n = 4) with the exception of the human study with Hank's receiver solution (n = 3). Th[a] is skin thickness; Per[b] is % skin permeation; Cum[c] is cumulative absorption in 24 h; Rp[d] is the maximum rate of skin permeation. R&H[e] specifies the receiver solution (Ringer's or Hank's) used in each study. Pool Parameters[f] specify the parameters used to create the pool donor solution including; DW (distilled water (100 mL)), CC (unlabelled cyanurate (55 ppm)), CL (chlorine (1.5 ppm) as well as the ^{14}C-cyanurate and ^3H_2O radiolabels that were present in all cases. An * indicates use of 1 μCi of ^{14}C-labelled CYA or DEET (otherwise 100 μCi ^{14}C-DEET were applied. In the finite dose study 0.1 μCi of ^{14}C-DEET was applied. Note that **DEET** indicated above in the pool parameters indicates a study with rat skin where ^{14}C-DEET was used in place of ^{14}C-cyanurate as detailed in the text. Note (**) that the thickness for Testskin^R was provided by Organogenesis Inc., Cambridge, MA.

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REFERENCES

Allen LM, Briggle TV, Pfaffenberger CD (1982) Absorption and excretion of cyanuric acid in long-distance swimmers. Drug Metabol Rev 13:499-516.

Bronaugh RL, Stewart RF, Simon M (1986) Methods for in vitro percutaneous absorption studies. VII: Use of excised human skin. J Pharm Sci 75:1094-1097.

Canelli E (1974) Chemical, bacteriological, and toxicological properties of cyanuric acid and chlorinated isocyanurates as applied to swimming pool disinfection. Amer J Public Hlth 64:155-162.

Datta PR (1979) Human exposure analysis of swimmers to chlorinated cyanuric acid via oral routes. Hazard Evaluation Division Report prepared for Special Pesticide Review Division, p1-5.

Downes CJ, Mitchell JW, Viotto ES, Eggers NJ (1984) Determination of cyanuric acid levels in swimming pool waters by u.v. absorbance, HPLC and melamine cyanurate precipitation. Water Res 18:277-280.

EPA (1988) Guidance for the reregistration of pesticide products containing chlorinated isocyanurates as the active ingredient. Document No. 540/RS-88-077.

Guy RH, Maibach HI (1984) Correction factors for determining body exposure from forearm percutaneous absorption data. J Appl Toxicol 4:26-28.

Inokuchi N., Sawamura R., Hasegawa A., Urakubo G. (1978) Distribution, percutaneous absorption and excretion of isocyanuric acid. Eisei Kagaku, 24:1-49.

Moody RP, Martineau P (1990) An automated in vitro dermal absorption procedure: I. Permeation of ^{14}C-labelled N,N-diethyl-m-toluamide through human skin and effects of short-wave ultraviolet radiation on permeation. Toxic in Vitro 4:193-199.

Moody RP, Ritter L. (1992) An automated in vitro dermal absorption procedure: II. Comparative in vivo and in vitro dermal absorption of the herbicide fenoxaprop-ethyl (HOE 33171) in rats. Toxic in Vitro 6:53-59.

Wester RC, Mobayen M, Maibach HI (1987) In vivo and in vitro absorption and binding to powdered stratum corneum as methods to evaluate skin absorption of environmental chemical contaminants from ground and surface water. J Toxicol Environ Hlth 21:367-374

Wojtowicz JA, Faust JP, Brigano FA (1984) Treatment of swimming pools, spas and hot tubs. Encyclopedia of Chemical Technology, 3rd edition. 24:427-441

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