RADIOISOTOPE STUDIES TO DETERMINE THE DISTRIBUTION OF REPELLENT (DEET) AND TOXICANT RESIDUES FROM TREATED CLOTH[1]

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ABSTRACT. Tests were conducted using radiolabeled deet (N, N-diethyl-m-toluamide), DDT (1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl)ethane), aldrin (1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene) and Thanite® (isobornyl thiocyanoacetate) to determine the distribution of these compounds from a treated stocking onto a protective nylon stocking or a filter paper substrate (representing the human arm) beneath the protective stocking. With DDT, aldrin and Thanite, about 0.01% (0.1 mg) of the total dose was recovered from the filter paper substrate. With deet, approximately 8 times that amount (0.8 mg) was recovered from the filter paper substrate. Amounts of residues found on the protective stocking were 2 to 5 times the amount found on the filter paper with all compounds except deet where less was found than on the filter paper.

Over the past 40 years there has been a continuing search for more effective personal-use repellents for application to skin or clothing to protect against biting insects and arthropods that are either pests or disease vectors. At the Insects Affecting Man and Animals Research Laboratory over 30,000 compounds have been considered for their potential use as repellents. Other groups, including industry, have tested some of the same materials. This testing and development research has led to the approval and use of several effective repellents either alone or in combination. Over the many years devoted to repellent development, the test methods used to discover repellent activity and develop the promising candidates for practical use (Linduska and Morton 1947; Schreck 1977) have been improved, but the basic approach has been the same, i.e., first a screening approach to determine repellent activity; then toxicological studies to permit safe application to skin or clothing for further testing and finally laboratory and field studies in simulated or actual problem situations. To insure the safety of such developmental programs only nonhazardous compounds of known structure or toxicity are considered. In addition to the toxicological studies on promising candidates prior to their direct application to skin or clothing, serological examinations are routinely conducted on individuals involved in the testing program.

Although a variety of methods of screening for clothing repellent activity have been proposed, the one most commonly used during this period involved exposing arms covered with treated cotton stockings to caged female mosquitoes (Schreck 1977). In this assay the center portion of the stockng with an area of 282 cm^2 (0.3 ft^2) is impregnated with an acetone solution of a candidate compound at the rate of 3.57 mg/cm^2 (total amount = 1 gm). After 2 hours (time allowed for acetone to evaporate), the treated stocking is pulled over the arm which is protected by an untreated nylon stocking between the arm and treated stocking. A cotton glove is worn over the

[1] This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture or the Department of Defense, nor does it imply registration under FIFRA as amended. Also, mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA or DoD.

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hand to protect it from mosquito bites and the arm is exposed in a cage of adult mosquitoes for 1 minute. The test is repeated after 24 hours and then weekly until 5 mosquito bites are received during a 1-minute exposure. The protective nylon stocking and the arm are washed with hot soapy water after each test. This method has been used for many years without evidence of candidate compound toxicity to the volunteers.

However, we did not have nor could we find any data on the distribution of residues on the protective stocking or the arm from compounds tested in this manner. The purpose of this study was to determine this distribution by the use of radioisotopes.

METHODS AND MATERIALS

Two methods were used in these studies. The first method was used to develop techniques and involved using a glass sleeve, 6.5–8.5 cm diam × 33 cm long or approximately the size of an arm, which was at first covered with a leather chamois to simulate the skin substrate. A protective nylon stocking (ladies' knee-high) and then a treated stocking were pulled over the chamois. Observations and preliminary tests indicated that the chamois was not a suitable substrate. The coarse surface of the chamois protruded through the protective nylon stocking and a yellow color leached from the chamois into the liquid scintillation solution reducing the efficiency of the radioactivity assay. Other materials were evaluated and the chamois was replaced by filter paper (Curtin #7760) for further tests. Thus, a test using glass sleeves involved a filter paper substrate covered by a protective nylon stocking and a treated stocking.

The second method involved the use of a human arm. The arm and hand were covered with a shoulder-length, vinyl, veterinary glove. Then the protected arm and hand were covered with filter paper over which the protective nylon and treated stocking were pulled. The treated stocking was left on for 2 minutes and then removed.

Very few radiolabeled compounds (and only in limited amounts) were available to us, but included deet (N, N-diethyl-m-toluamide) and the toxicants DDT (1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl)ethane), aldrin (1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene), and Thanite® (isobornyl thiocyanoacetate). Deet is a liquid with some volatility and is the general, all-purpose repellent in use. DDT is a toxicant and, therefore, never considered as a repellent. However, it is a solid with essentially no volatility and completely different chemically. DDT was used for many years directly applied to humans for louse and scabies control. Aldrin and Thanite were used primarily to develop methods since aldrin is a solid and Thanite is an oily liquid, with some repellent qualities.

Deet, DDT and Thanite were carbon-14 labeled while aldrin was chlorine-36 labeled. All compounds had purity >98%. The specific activity of the compounds was: deet – 13.6 mCi/m mole, DDT – 4.35 mCi/m mole, Thanite – 37 mCi/g and aldrin 110 μ Ci/g. The radiolabeled compounds were mixed with non-radioactive samples of the same chemical and applied to the treated stockings as described above so that 1.0 g was spread over the 282 cm^2 middle area. After exposure on either the glass sleeve or arm, the filter paper and the protective nylon stocking were extracted for assay of radioactivity in a Packard Tri-Carb® liquid scintillation spectrometer. Sixty to 80 ml of toluene were used to rinse the filter paper or the nylon stocking and then evaporated to 10 to 15 ml for assay of radioactivity. Samples of filter paper and nylon hose used in the tests were assayed after extraction to make certain all radiolabeled material had been removed. The filter paper in the arm tests was divided into 2 areas, one over the arm and one over the hand and analyzed separately. The amount of chemical recovered

from the filter paper and nylon stocking was calculated after correcting for control filter paper and nylon stockings.

RESULTS AND DISCUSSION

The results of these analyses are presented in Table 1. In our preliminary studies using the glass sleeve there was little difference in the amount of DDT, Thanite or aldrin found on the filter paper substrate, i.e. ca. 0.1 mg or 0.01% of the total dose. Also in preliminary studies there was little difference in the amount of DDT or Thanite found on the filter paper substrate when it was placed over the human arm, i.e. ca. 0.1 mg or 0.01% of the total dose. Amounts of these chemicals found in the protective nylon stocking were more variable, but quantitatively similar and only 2 to 5 times the amount recovered from the filter paper. Thus, our preliminary tests showed little difference in the amounts of compounds (DDT, Thanite and aldrin, all with little volatility) transferred from the treated cloth to the protective stocking and filter paper.

In our final tests on the human arm, the amount of deet found on the protective stocking was ca. 3 times greater than the DDT found, but the amount of deet recovered from the filter paper was about 10 times that of DDT. With a liquid, such as deet, with some degree of volatility, it appears that some mechanism other than physical contact, i.e. volatilization, played a role in the transfer. The use of a disposable glove placed over the hand can be used to prevent the amount of residues getting on the hand, thus reducing the total residues per test up to 50%.

The arm area used in the screening procedure is ca. 708 cm^2, thus with deet residues of 0.788 mg per test the per unit area exposure was 1.111 $\mu g/cm^2$. Other compounds gave residues of about 0.1 mg per test, thus the per unit area of exposure was 0.14 $\mu g/cm^2$.

To put these data into perspective as to toxic doses in terms of LD-50 dermal exposure, assuming a weight of 80 kg for an average person conducting the test, the deet exposure would be 0.788 mg per test, or 0.01 mg/kg (ppm). With the deet exposure in terms of its LD-50 dermal value of 10,000 mg/kg, over 1,000,000 tests would be required to reach the LD-50 level if the amount were cumulative and if none were removed by washing after tests. These figures illustrate only a minimal exposure with the standard test method in use and the unlikely occur-

Table 1. Average amount of test chemical found on filter paper substrate and the protective nylon stocking using a glass sleeve and the protected arm of a human.[a]

Chemical	Test method	No. of tests	Avg. mg chemical recovered: Protective nylon hose	Filter paper over hand	Test area filter paper	% on test area filter paper of total applied
Thanite	Glass sleeve	8	0.502	—	0.103	0.010
	Arm[a]	2	0.802	0.096	0.103	0.010
Aldrin	Glass sleeve	2	0.500	—	0.145	0.014
DDT	Glass sleeve	2	0.214	—	0.107	0.011
	Arm[a]	2	0.155	0.075	0.073	0.007
Deet	Arm[a]	10	0.511 (SE±0.2151)	0.280 (±0.0375)	0.788 (±0.055)	0.079

[a] Arm and hand protected with a shoulder-length veterinary glove.

rence of overexposure, particularly since compounds selected for clothing repellent tests are also screened for hazardous compounds, toxicants and chemical structure prior to testing.

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EVALUATION OF THE COMBINED EFFECTS OF METHOPRENE AND THE PROTOZOAN PARASITE *ASCOGREGARINA CULICIS* (EUGREGARINIDA, DIPLOCYSTIDAE), ON *AEDES* MOSQUITOES[1]

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ABSTRACT. Mortality rates for test populations of *Aedes aegypti* were significantly increased with increases in concentrations of methoprene in the larval rearing media from 1.0 ppb (28% average mortality) to 10 ppb (84% average mortality). The mortality rates were not significantly changed when the protozoan parasite, *Ascogregarina culicis*, was used in combination with either concentration of methoprene against *Ae. aegypti* larvae. In contrast, mortality rates for *Ae. epactius* were not only significantly increased with increases in methoprene concentrations from 0.001 ppb (13% average mortality) to 0.01 ppb (53% average mortality), but also, the mortality rates at each IGR concentration were significantly higher when *Ae. epactius* larvae were first exposed to sporocysts of *A. culicis* and then to the IGR. Average mortality rates in this latter case ranged between 73 to 82%. The combined effects of *A. culicis* and methoprene on the mortality rates for *Ae. epactius* appear to be additive. Methoprene appears to have no significant effect either on the infectivity of the sporocyst stage of *A. culicis* or on the level of parasitism that can be established by this parasite in *Ae. aegypti* and *Ae. epactius* populations.

A common approach to integrated control involves the utilization of insecticides and biological control agents in a supplementary and complementary manner. Naturally occurring populations of biological control agents may be used or they may be artificially introduced into a habitat in conjunction with insecticide application, the critical factors being the compatibility of the biological control agent and the chemical insecticide, and the time of application of each. Therefore, preparatory to the integration of any biological and chemical control agents, a thorough study of the combined effects and compatibility of the control agents must be undertaken.

The purpose of this study was to conduct a laboratory investigation of the potential effects that the introduction of

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