AD-A125 214 DERMAL PENETRATION AND DISTRIBUTION OF 14C-LABELED NN-DIPROPYLCYCLOHEXANE..(U) ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GROUND MD H L SNODGRASS

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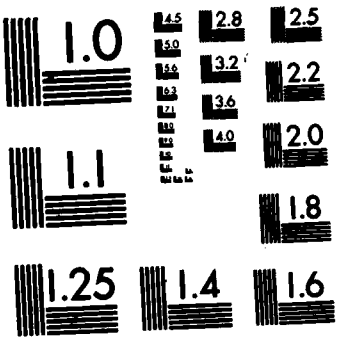

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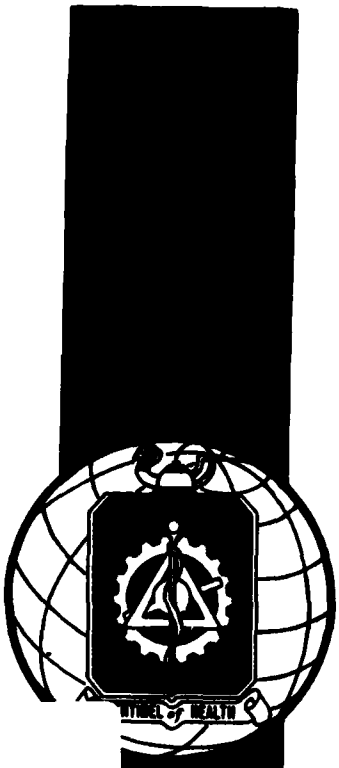

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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

PHASE 2
DERMAL PENETRATION AND DISTRIBUTION OF ^{14}C-LABELED
N,N-DIPROPYLCYCLOHEXANE CARBOXAMIDE, AI3-36326
STUDY NO. 75-51-0233-83
MAY-OCTOBER 1982

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

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9. PERFORMING ORGANIZATION NAME AND ADDRESS: US Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD 21010	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS:
11. CONTROLLING OFFICE NAME AND ADDRESS: Commander, US Army Health Services Command, Fort Sam Houston, TX 78234	12. REPORT DATE: May-October 1982
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18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

AI3-36326	Penetration	N,N-Dipropylcyclohexane
Dermal	Rabbit	
Dog	Radiolabeled	
Execretion	Repellent	
Intraveuous	Tissues	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The candidate insect repellent N,N-dipropylcyclohexane carboxamide was assessed for absorption potential in rabbits and dogs by monitoring radiocarbon in excrets for 7 days following a single application. In both species, absorption occured primarily in the first 24 hours and measured 26 percent of the applied dose in rabbits and 12 percent in dogs through 7 days. No bioaccumalation in tissue specimens was noted at necropsy by this route. Absorption in man should be less than 10 percent of the applied dose.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Snodgrass/jg/AUTOVON
584-3980

REPLY TO ATTENTION OF
HSHB-OT-P/WP

1 MAR 1983

SUBJECT: Phase 2, Dermal Penetration and Distribution of 14C-Labeled N,N-Dipropylcyclohexame Carboxamide, AI3-36326, Study No. 75-51-0233-83, May-October 1982

Executive Secretary
Armed Forces Pest Management Board
Forest Glen Section, WRAMC
Washington, DC 20012

EXECUTIVE SUMMARY

The purpose, essential findings and conclusions of the inclosed report follow:

a. Purpose. N,N-Dipropylcyclohexame Carboxamide (carboxamide) is a promising insect repellent. The potential for skin pentration and bodily distribution was assessed in rabbits and dogs using the radiolabeled (ring 14C) chemical.

b. Essential Findings. Absorption of topically applied carboxamide totaled 26 percent of the applied dose after 7 days in rabbits and 12 percent in dogs. Urinary elimination accounted for nearly all of the absorbed chemical and occurred within the first 24 hours. Tissue specimens collected at necropsy were void of detectable radioactivity. Following a single intravenous injection of carboxamide to both species, radiocarbon was rapidly eliminated in urine and measured 72 and 84 percent of the injected dose, respectively, for rabbits and dogs. At necropsy, radioactivity in tissues showed a slight but consistent localization in adrenal glands, bone marrow, spleen and lungs.

c. Conclusions. Based on the current study and others, dermal absorption of carboxamide in man would be expected to be lower than that demonstrated in animals, probably less than 10 percent of the applied dose. No potential for tissue binding has been noted after topical application. The slight localization of radiocarbon in tissues following intravenous injection, however, remains of interest.

FOR THE COMMANDER:

JOHN W. CUTTING, M.D.
Colonel, MC
Director, Occupational and Environmental Health

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as (5 cy)

CF:
HQDA (DASG-PSP) wo incl
Cdr, HSC (HSPA-P)
Comdt, AMS (HSHA-IPM)
Dir, Advisory Ctr Div Tox, NRC (2 cy)
USDA, ARS, Southern Region

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

REPLY TO ATTENTION OF
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PHASE 2
DERMAL PENETRATION AND DISTRIBUTION OF ^{14}C-LABELED
N,N-DIPROPYLCYCLOHEXANE CARBOXAMIDE, AI3-36326
STUDY NO. 75-51-0233-83
MAY-OCTOBER 1982

1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the Department of Agriculture, Agricultural Research Science and Education Administrations; titled Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, Armed Forces Pest Control Board, [illegible] November 1979, subject: Toxicological Testing of Candidate Repellent Compounds.

c. Letter, Armed Forces Pest Control Board, 9 March 1979, subject: Prioritization of Promising Insect Repellent Compounds Requiring Toxicological Testing.

2. REFERENCES.

a. Proposed Guidelines for Registering Pesticides in the United States; Hazard Evaluation: Human and Domestic Animals, 43 Federal Register (FR) 37336, 22 August 1978.

b. Toxicology Division Standing Operating Procedure, Radioisotope Studies, US Army Environmental Hygiene Agency (USAEHA), April 1981.

c. A bibliography is listed as the Appendix.

3. SPONSOR. Armed Forces Pest Management Board (AFPMB), Washington, DC.

4. BACKGROUND. AI3-36326 (carboxamide) is a candidate insect repellent proposed for use by military personnel and is currently undergoing toxicological evaluation in the Toxicology Division, USAEHA. Its proposed use is as a topically applied solution on human skin and/or on clothing by spray application. The rabbit has been suggested as the animal of choice for dermal testing (reference 2a) owing to its tendency towards maximal absorption of topically applied chemicals in comparison to other animal species. The Beagle dog has also shown promise as an animal model for dermal testing because it more closely resembles man's absorption kinetics.[1]

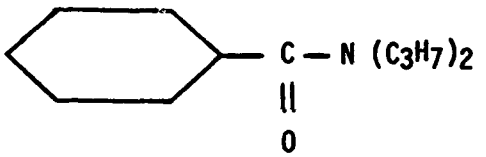
5. PURPOSE. The purpose of this study was to quantitate the rate of penetration of ^{14}C-labeled carboxamide through the intact skin of rabbits and dogs. The dermal absorption and bodily distribution of carboxamide was assessed by monitoring the radioactivity in excreta for 7 days and selected tissues at necropsy. The methodology (reference 2b) has been previously documented.

6. MATERIALS.

a. Radiolabeled N,N-di-n-Propyl cyclohexane carboxamide [ring-^{14}C (U)]; Lot 1193-219. Radioactivity: 10.7 millicuries; weight 110.6 milligrams; specific activity: 20.43 millicuries/millimole; purchased from New England Nuclear, Boston, Massachusetts. Radiochemical purity was 98 percent.

b. Unlabeled N,N-dipropyl cyclohexane carboxamide, AI3-36326, was supplied by Terrence P. McGovern, Ph.D., Organic Chemical Synthesis Laboratory, Agricultural Research Center, US Department of Agriculture, Beltsville, Maryland 20705.

c. The chemical structure of AI3-36326 (carboxamide) is as follows:



7. ANIMALS.

a. Six male New Zealand White rabbits, weighing between 2.0 and 2.5 kg, were purchased from Dutchland Laboratories, Denver, Pennsylvania. Animals were individually housed in Wahmann stainless steel metabolism cages and received food (Aberdeen 09 Rabbit Ration, Zeigler Brothers, Inc., Garners, Pennsylvania) and water ad libitum.

b. Six male Purebred Beagle dogs, 22 months old, were selected from USAEHA kennel stock. These animals were originally purchased from Laboratory Research Enterprises, Inc., Kalamazoo, Michigan. Dogs were individually housed in Wahmann stainless steel metabolism cages and received food (Respond 2000, ProPet, Inc., Syracuse, New York) and water ad libitum.

8. METHODS.

a. The ^{14}C-labeled carboxamide was initially diluted with absolute ethanol (Warner Graham Co., Cockeysville, Maryland). A further dilution for animal administration was made in 95 percent ethanol. In the rabbit tests

unlabeled carboxamide was added to increase the mass concentration to 369.2 ug/mL to satisfy our target application rate of 4 ug/cm^2.

b. Three rabbits received a single intravenous injection of ^{14}C carboxamide to assure that systemic elimination of the chemical was measurable in excreted urine. Each animal received a radioactive dose of 6.42 microcuries (uCi) and chemical mass of 110.8 micrograms (ug) injected into the marginal ear vein. The 0.3 mL volume was introduced into a slow saline infusion (2.0 mL) to prevent the necrotizing effect of alcohol on the vein lumen.

c. The remaining three rabbits received the chemical topically. The mid-lumbar area of the animal's back was clipped free of hair and a 27.7 cm^2 area demarcated with petrolatum to contain the chemical while the alcohol diluent dried. The applied dose was the same as for the intravenous rabbits. The application rate was 4 ug/cm^2 for each animal. The application area was then covered with a nonocclusive patch (reference 2b) which protected the area without contacting the radiochemical. The patch was changed after 24 hours and a new one affixed.

d. Dogs also received the carboxamide as a single intravenous or topical dose although the solution concentration differed from the rabbit tests. Three dogs were treated intravenously with a 0.5 mL volume containing 7.5 uCi of radioactivity and chemical mass of 77.4 ug. Injection was made into the cephalic vein via a saline infusion. The remaining three dogs received the same dose topically contained in a 19.4 cm^2 area or application rate of 4 ug/cm^2. The area was covered with a nonocclusive patch and changed at 24 hours.

e. Dogs receiving intravenous carboxamide were monitored following injection at timed intervals through 24 hours to assess disappearance rates ($t_{1/2}$) of radiocarbon from circulating blood. Blood specimens were collected, centrifuged, and 0.2 mL aliquots of plasma added to 15 mL of PCS®II and radioactivity measured. A semilog plot of radiocarbon levels versus time was constructed using the "stripping" technique.[2] The $t_{1/2}$ was determined for the rapid distribution phase.

® PCS is a registered tradename of Amersham Corp, Arlington Heights, Illinois. Use of trademarked names does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

f. Immediately after administration of the test chemical, each animal was returned to its cage. Urine was collected and measured daily through the 7-day test period. Aliquots (0.2 mL) were combined with 15 mL of PCS II scintillation cocktail and radioactivity measured using a Model LS9000, Beckman Liquid Scintillation Counter. Dog feces were collected daily, weighed and combined with 3 volumes of methanol. After mixing for 24 hours, aliquots were measured for radioactivity.

g. At the end of the study period, animals were euthanized and representative tissue and fluid specimens collected and measured for radiocarbon. Specimens included liver, lung, kidney, spleen, heart, brain, adrenal glands, urinary bladder, muscle, bone, skin, fat, thyroid glands, testes, bone marrow, blood, and bile. Radioactivity was assessed following oxidation of each 0.25 to 0.45 g specimen to $^{14}CO_2$ using a Hewlett-Packard Biological Materials Oxidizer.

h. The unabsorbed fraction of topically applied carboxamide was quantitated by extracting, in 95 percent ethanol, the nonocclusive patches and the excised skin from the application site. Extract fractions were combined with the scintillation cocktail and radioactivity counted.

i. Urinary and fecal excretion of radiocarbon was quantitated over a 7-day study period. Excretion rates for each day's collection were calculated as the percent recovery of the initial injected or applied dose. Calculations for tissue specimens collected at necropsy were based on counts (c.p.m.) per specimen and reported as d.p.m./gram of wet tissue following correction for efficiency.

9. RESULTS.

a. The bioelimination of ^{14}C-labeled carboxamide following a single intravenous injection to rabbits and dogs was rapid. Over 70 percent of the injected dose was recovered in urine during the first 24 hours in both species (Tables 1 and 2). In rabbits, less than 1 percent was eliminated during the remaining 6 days for a total cumulative recovery of 72 percent. In dogs, 6 percent of the injected dose appeared in urine beyond the first day for a total of 83 percent through the 7-day test. The disappearance of circulating radiocarbon from dog blood ($t_{1/2}$) took about 60 minutes.

b. The bioelimination of injected carboxamide appearing in dog feces measured 5 percent of the given dose through 5 days. No radioactivity was noted beyond that period. This value, combined with that contained in urine, totaled 87 percent recovery of the injected chemical.

Phase 2, Study No. 75-51-0233-83, May-Oct 82

TABLE 1. FATE OF ^{14}C-LABELED CARBOXAMIDE FOLLOWING A SINGLE INTRAVENOUS INJECTION TO RABBITS

URINARY EXCRETION (n = 2)

Day	% of Dose
1	71.6 ± 1.5
2	0.4 ± 0.1
3	0.1 ± 0.1
4	0.1 ± 0.1
5	0.1 ± 0.0
6	0.0 ± 0.0
7	0.1 ± 0.1
Total	72.4 ± 1.3

TISSUE DISTRIBUTION AFTER 7 DAYS (n = 3)

Specimen	DPM/gram	Responses*
Adrenal Glands	446 ± 76	3/3
Liver	231 ± 78	3/3
Bone Marrow	191 ± 25	3/3
Spleen	170 ± 216	3/3
Lungs	43 ± 13	3/3
Kidneys	22 ± 30	1/3
Fat	20 ± 29	1/3
Blood	11 ± 1	2/3

* Specimens containing detectable activity vs animals monitored

Phase 2, Study No. 75-51-0233-83, May-Oct 82

TABLE 2. FATE OF ^{14}C-LABELED CARBOXAMIDE FOLLOWING A SINGLE INTRAVENOUS INJECTION TO DOGS

PERCENT OF INJECTED DOSE (n = 3)

Day	Urinary Excretion	Fecal Excretion
1	77.0 ± 7.1	2.7 ± 0.2
2	5.1 ± 3.6	1.7 ± 1.6
3	0.9 ± 0.7	0.3 ± 0.2
4	0.2 ± 0.2	0.1 ± 0.1
5	0.1 ± 0.2	0.1 ± 0.1
6	0.1 ± 0.1	<LLD
7	<LLD	<LLD
Total	83.4 ± 3.5	4.9 ± 0.6

LLD - Lower Limit of Detectability

TISSUE DISTRIBUTION AFTER 7 DAYS (n = 3)

Specimen	DPM/gram	Responses*
Adrenal Glands	63 ± 8	3/3
Kidneys	79 ± 79	2/3
Liver	46 ± 47	2/3
Fat	24 ± 17	2/3
Blood	<LLD	

* Specimens containing radioactivity vs animals monitored

c. Tissue specimens collected from rabbits and dogs at the end of the 7-day intravenous test showed a consistent localization of radioactivity in the adrenal glands and is reported in Tables 1 and 2. Lesser amounts were registered in liver, kidneys, spleen, lungs, bone marrow, and fat specimens. Blood samples collected at necropsy were generally without significant circulating radiocarbon, registering only 1 or 2 counts above the LLD.

d. Absorption of topically applied carboxamide to rabbits, as measured by urinary excretion, totaled 26 percent of the applied dose (Table 3). Eighty-five percent of the absorbed radiocarbon was recovered in the first 24 hours. Unabsorbed carboxamide was primarily recovered from the nonocclusive patches which trapped exfoliated skin and probably some volatilized chemical. These measured 24 percent of the applied dose. An additional 9 percent was recovered from skin at the application site (Table 3).

TABLE 3. FATE OF ^{14}C-LABELED CARBOXAMIDE FOLLOWING A SINGLE TOPICAL APPLICATION TO RABBITS

PERCENT OF APPLIED DOSE (n = 3)

Day	Urinary Excretion
1	21.8 ± 3.9
2	2.5 ± 0.8
3	0.7 ± 0.1
4	0.2 ± 0.0
5	0.1 ± 0.0
6	0.2 ± 0.1
7	0.1 ± 0.1
Total	25.6 ± 3.6

TOTAL PERCENT RECOVERY (n = 3)

Urine	Skin Application Site	Patches Day 1	Patches Day 7	Total Recovery
25.6 ±3.6	9.4 ±10.1	17.7 ±8.6	6.0 ±3.9	58.7 ±8.1

e. Absorption of topical carboxamide to dogs measured 13 percent of the applied dose through 7 days. Twelve percent was recovered in urine, and about 1 percent from feces (Table 4). Absorption in dogs was slightly slower compared to rabbits but was essentially complete after 3 days. Unabsorbed radiocarbon recovered in nonocclusive patches and skin at the application site totaled 48 percent of the applied dose (Table 4).

TABLE 4. FATE OF ^{14}C-LABELED CARBOXAMIDE FOLLOWING A SINGLE TOPICAL APPLICATION TO DOGS

PERCENT OF APPLIED DOSE (n = 3)

Day	Urinary Excretion	Fecal Excretion
1	5.8 ± 0.6	0.4 ± 0.2
2	4.0 ± 0.9	0.4 ± 0.1
3	1.2 ± 0.2	0.2 ± 0.0
4	0.3 ± 0.1	0.1 ± 0.0
5	0.2 ± 0.0	0.1 ± 0.1
6	0.2 ± 0.0	<LLD
7	0.2 ± 0.1	<LLD
Total	11.9 ± 1.4	1.2 ± 0.1

TOTAL PERCENT RECOVERY (n = 3)

Urine	Feces	Skin Application Site	Patches Day 1	Patches Day 7	Total
11.9 ±1.4	1.2 ±0.1	2.5 ±0.9	40.1 ±6.3	5.2 ±1.5	60.9 ±5.5

f. No significant radiocarbon was detected in tissues from rabbits or dogs 7 days after topical application.

10. DISCUSSION.

a. The clearance of ^{14}C-labeled carboxamide was rapid from both the rabbits and dogs by the renal elimination pathway following intravenous injection. Radiocarbon appearing in urine after a topical application should, therefore, accurately reflect absorption potential in these species.

b. Volatilized carboxamide from the site of topical application probably accounts for the unrecovered radioactivity in both test species. It is unlikely that any significant chemical would be eliminated in expired air.

c. Radiolabeled carboxamide appearing in dog feces may not accurately reflect enterohepatic involvement. Some unavoidable contamination of feces by radioactive urine occurs through the design of the metabolism cages and may in fact contribute all of the observed radiocarbon. Further, radioactivity appearing in feces does not usually correlate with urinary excretion patterns. As a rule, urinary radioactivity following administration of radiochemicals to animals peaks within 24 hours. Maximum radiocarbon appearing in feces is often delayed, generally to 48 hours.

d. Of interest, is the slight (0.3 to 3.5 ppb/gram) but consistent localization of radiocarbon in the adrenal glands of both dogs and rabbits following a single intravenous injection of labeled carboxamide. This would suggest a first-pass type of tissue binding by the parenterally delivered chemical. To the contrary, animals treated topically showed no affinity for binding in any internal tissue. Biotransformation at the epidermal level or the absorption rate limiting effect of the skin barrier could account for this. From a safety standpoint [illegible] internal organ [illegible]biting localized radioactivity 1 week after compound administration, particularly extrahepatic tissue, must be considered a presumptive site of chemical-related toxicity.[3] However, within the intended use of carboxamide as a topical repellent, the potential for tissue bioaccumulation appears minimal.

e. One rabbit, parenterally treated with ^{14}C carboxamide, was not included in averaging excreted radioactivity in the urine. Considerable seepage occurred at the injection site immediately following dosing, hence, the total radiocarbon dose could not be assured. This was confirmed by a reduced (25 percent) radiocarbon content in urine 24 hours later when compared to the other test animals. Interestingly, tissue deposition data was consistent with the other test animals and was included.

11. CONCLUSION. Within the confines of the study there appears little hazard associated with the intended use of carboxamide as a topical insect repellent. Absorption in man would be expected to be lower than observed in dogs, probably less than 10 percent of the applied dose. Urinary excretion is the primary elimination pathway of absorbed chemical and is nearly complete within 24 hours. No potential for bioaccumulation in tissues has been noted following dermal application. Concurrent studies (in progress), using unlabeled carboxamide dermally in rabbits, have further confirmed the absence of toxic effects beyond local irritation at levels to 714 mg/kg for 15 days.

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APPENDIX

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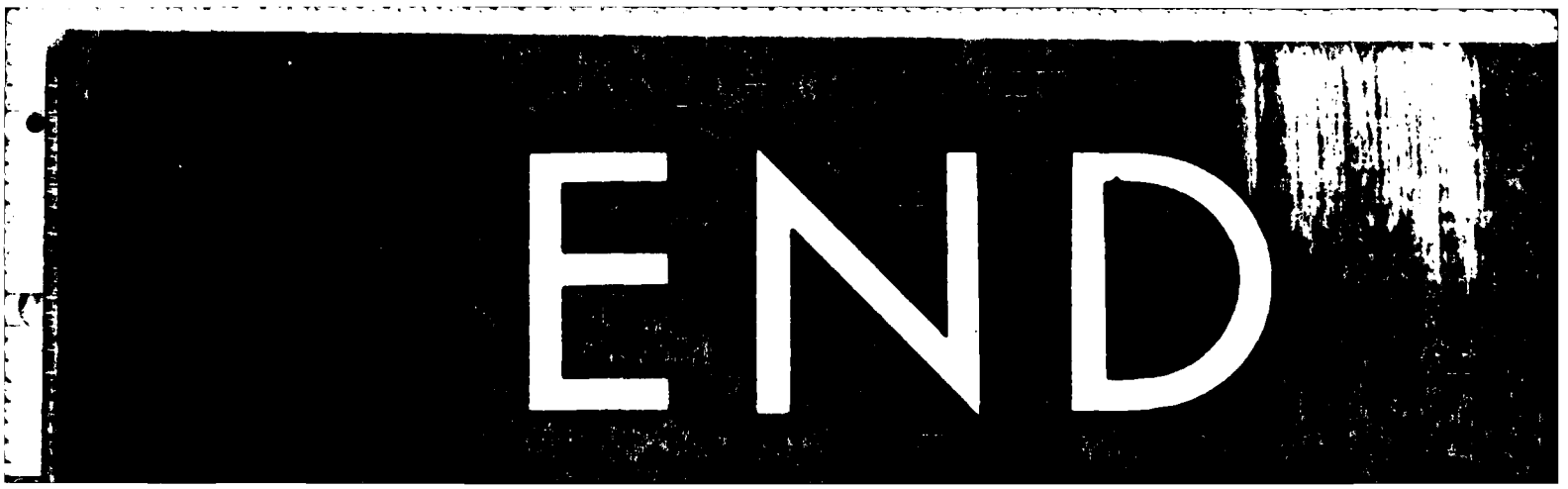
3. Goorod, J. W., "Covalent Binding in Drug Toxicity," Testing for Toxicity (J. W. Goorod, ed.), pp 77-93, Taylor and Francis LTD, London (1981).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