Methods I and II were very close. The total sugar values from the Hassid method also compared favorably with those obtained by either of the new techniques. It can be seen that the Hassid values for total sugar were slightly higher. In the case of the stems, the reducing sugar values from the Hassid method were closer to glucose plus fructose values in Method II than the corresponding values in Method I. However, with the leaves, there was no regular deviation in any of the three methods.

Several fruits and vegetables were analyzed using both Methods I and II. A comparison of the results from the two methods of testing is shown in Table IV. In general, the agreement between the two analyses was good. The total sugars were somewhat higher in Method II. This is to be expected since anthrone will react with any minor sugars or glycosides present in the extract. However, ascorbic acid and other non-sugar-reducing substances such as the plant polyphenols do not interfere with the anthrone analysis.

Both methods are rapid and gave reproducible results in the absence of interfering substances. Especially important was the complete removal of ascorbic acid before the glucose oxidase method was used. The effect of ascorbic acid on the analysis for glucose is shown in Table V. The samples were analyzed immediately and also after standing for 24 hours at 21° C. The last two columns show how the serious effects of ascorbic acid may be eliminated by using ascorbic acid oxidase. Even at 80 μg. of ascorbic acid per ml. the analyses were good when ascorbic acid oxidase was used. It is interesting to note the change of absorbance by using glucose oxidase when the samples were allowed to stand for 24 hours. All the ascorbic acid had disappeared by this time (except in the sample in which 80 μg. per ml. were added) and the absorbance increased. In all cases (except No. 5), the addition of ascorbic acid caused an increase in the absorbance without the addition of glucose oxidase. For these reasons, it appears that if ascorbic acid is present, the dilutions from the ethanolic plant extract should not be made until immediately before the analysis.

Table VI. Elimination of Ascorbic Acid (AA) with Activated Carbon Solution Analyzed after Standing 24 Hours at 21° C.

Sample	Treatment	Absorbance
1	100 μg. per ml. glucose	0.258
2	100 μg. per ml. glucose + 10 μg. AA	0.299
3	100 μg. per ml. glucose + 40 μg. AA	0.398
4	100 μg. per ml. glucose + carbon (50 mg. per 100 ml. soln.)	0.260
5	100 μg. per ml. glucose + 10 μg. AA + carbon	0.268
6	100 μg. per ml. glucose + 40 μg. AA + carbon	0.295

Activated carbon has also been found suitable for removal of ascorbic acid. Table VI gives the results of treatment with carbon to remove ascorbic acid before glucose analysis. Samples 2 and 3 had a high absorbance when analyzed after standing 24 hours. Treatment with carbon removed most of the ascorbic acid from sample 5 (10 μg. of ascorbic acid) but failed to remove the ascorbic acid from sample 6 (40 μg. of ascorbic acid), which contained a larger amount of ascorbic acid. Carbon itself had no effect on the glucose content (sample 4). Apparently carbon is not as suitable as ascorbic acid oxidase for removing ascorbic acid, particularly at the higher concentrations, at which residual hydrogen peroxide interferes.

In samples containing small amounts of sugars, the dilutions from the alcoholic extracts may be so small that the alcohol concentration is high enough to interfere with the invertase reaction. In this case, Method II may be preferred over Method I. In general, Method I gives greater specificity for free fructose and sucrose because of the possible error in total sugar determination using anthrone. If this higher degree of specificity is not required, then Method II is satisfactory.

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ANIMAL METABOLISM OF PLANT REGULATORS

Metabolism of 3-Amino-1,2,4-triazole-5-C^{14} by Rats

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THE GROWTH regulating effects exerted by 3 - amino - 1,2,4 - triazole (ATA) on plants have attracted considerable interest in recent years. This chemical was first reported to cause leaf abscission, chlorosis, and growth inhibition in cotton (*2, 3*), and similar effects on many other plant species have subsequently been reported. Pyfrom *et al.* (*9*) reported the inhibitive effect of ATA on plant catalase activity and the depression of chlorophyll synthesis. Heim, Appleman, and Pyfrom (*4*) found that catalase activity in rat liver and kidney was reduced in animals injected with ATA. The blood catalase and hemoglobin of the treated animals remained normal. The purpose of the present investigation was to characterize excretory patterns and to study tissue residues following oral administration of 3-amino-1,2,4-triazole-5-C^{14} to the rat.

Materials and Methods

Several trials were conducted with adult rats of the Wistar strain (4 to 6

3-Amino-1,2,4-triazole-5-C^{14} (ATA) was fed to rats at a dose of 1 mg. per rat, and the expired air, urine, feces, internal organs, and tissues were analyzed for radioactivity. Only insignificant traces of C^{14} were found in the expired air during the 3-day period following dosing. During the first 24 hours, from 70.0 to 95.5% of the radioactivity was found in the urine, which contained two radioactive metabolites in addition to ATA. The feces contained a small but variable amount of activity. After absorption, ATA was distributed throughout most of the body tissues. The maximum radioactivity in all tissues was generally reached within 1 hour, and started to decrease after 3 to 4 hours following dosing. Elimination of ATA from all tissues was fast. The alcohol extracts of all tissues, with the exception of liver and kidney, contained mainly ATA residue with a trace of metabolite-1. Liver was the site of metabolite-1 formation, and the rate of elimination of this metabolite from liver and kidney was much slower.

months of age) to determine the accumulation pattern and excretory pathways of orally administered 3-amino-1, 2,4-triazole-5-C^{14} (ATA-5-C^{14}). The ATA-5-C^{14}, 0.97 mc. per mmole (purchased from Tracerlab, Waltham, Mass., and containing no isotopic impurity, as revealed by paper chromatography), was dissolved in 25% ethanol solution at a concentration of 1 mg. per ml. and refrigerated before use. The isotope was administered via stomach tube with dosage as indicated. After dosing, each rat was placed in a glass metabolism cage where, at intervals, feces, urine, and expired carbon dioxide were collected separately. The carbon dioxide produced was continuously trapped in a gas absorber containing 2*N* NaOH solution which was changed periodically at predetermined intervals. The carbonate was precipitated as $BaCO_3$, filtered through a glass-fiber filter paper, weighed, and counted. The radioactivity of the $BaCO_3$ was corrected for self-absorption and background. Urine samples were collected daily. After the urine was drained, the separator was rinsed thoroughly first with alcohol and then with water. Both urine and washings were combined and made to volume. Duplicate aliquots of each urine sample were plated directly on stainless steel cupped planchets, dried, and their radioactivities counted. Each feces sample was extracted with 50 ml. of ethanol, and 0.5-ml. aliquots of ethanol extract were plated, dried, and counted. A thin mica end window (1.4 mg. per sq. cm.) G-M counter with a manual sample changer was utilized in making all radioactivity measurements. High activity samples were counted to 2% error, while low activity samples were counted for 30 minutes each.

In the first series, the rats were sacrificed at a predetermined period of 2 to 6 days after receiving a single oral dose. The second series, consisting of 10 male rats, were sacrificed 30 minutes to 72 hours after dosing. Various tissues were carefully separated and freeze-dried. The dry tissues were weighed and finely ground. Duplicate aliquots of 0.1 gram of dry sample were weighed in planchets and counted directly, and additional aliquots were oxidized by a wet combustion technique (*7*) to determine the conversion factor between counting radioactivity as $BaCO_3$ and as a dry sample. The relationship between direct counting of urine or feces extract and counting of these liquid samples after conversion to $BaCO_3$ was also established in a similar manner. All activities of the dry tissues and other samples were converted to $BaCO_3$ counting.

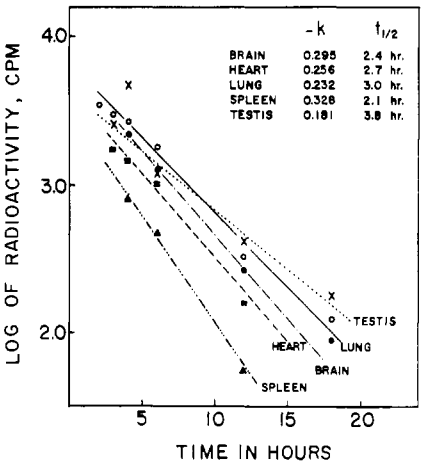

Figure 1. In vivo kinetics of ATA-5-C^{14} elimination in the brain, heart, lung, spleen, and testis of rats following a single oral dose of ATA-5-C^{14}

To define the chemical nature of the radioactivity of the urine and the alcohol extract of feces, small aliquots of these samples were chromatographed one dimensionally on Whatman No. 1 filter paper using isopropanol–NH_4OH–H_2O (10:1:1) as developing solvent. Dry tissues containing sufficient radioactivity were exhaustively extracted with ethanol for 72 hours in a Soxhlet apparatus. The alcohol extract was concentrated to small volume, shaken several times with a small amount of isoöctane to remove fatty material, and then subjected to paper chromatographic separation. Both direct scanning and radioautographic techniques were utilized.

Results

From the study of eight rats, insignificant traces of C^{14} were found in the expired air during a 3-day period immediately following dosing. This observation indicated that ATA-5-C^{14} was not completely oxidized. Between 70.0 and 95.5% of the radioactivity was found in the urine during the first 24-hour period (calculated from data in Table I). The feces contained a small but variable amount of activity, thus indicating that this compound was almost completely absorbed from the gastrointestinal tract. Fecal radioactivity, in some cases, due to incomplete separation, was contaminated from the urine. The average radioactivity excreted in the feces of six rats (excluding No. 15 and 16 because of contamination from urinary radioactivity) during the first 24-hour period was 3.9% and decreased to 0.2% in the next 48 hours. Total recoveries of radioactivity in both urine and feces were between 80 and 104%, with an average value of 92.1%.

A very small amount of radioactivity was found in most internal organs and tissues from rats sacrificed after the third day. The liver was the only organ that still contained significant amounts of radioactivity which decreased as time progressed. The average value for the C^{14} remaining in the liver after 3 days was 0.68% of the administered dose, with a range from 0.42 to 0.98% for male rats and 0.62 and 0.74% for female rats. Paper chromatographic and radioautographic results revealed that ethanol extracts of rat liver from animals receiving ATA-5-C^{14} contained two major radioactive spots. One spot (R_F 0.40) was unchanged ATA while the other was metabolite-1 (R_F 0.00). The formation of radioactive metabolite-1 and the disappearance of ATA in liver from animals which were sacrificed at various times after receiving ATA-5-C^{14} are presented in Table II. No free ATA was found in liver extract after 24 hours. Very little radioactivity remained in either the stomach or intestine, but higher activity was generally found in the intestine. In

Table I. Pathways of C^{14} Excretion (C.P.M.) of Adult Rats Following a Single Oral Administration of 1.0 Mg. of ATA-5-C^{14} [a]

Time after Dosing, Days	No. 10, M [b]		No. 11, M		No. 13, M		No. 15, M		No. 9, F		No. 12, F		No. 14, F		No. 16, M	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
1	1,350,000	64,000	1,240,000	22,300	1,147,000	40,000	1,091,500	340,500	1,490,000	84,700	1,329,000	65,400	1,305,900	85,700	1,292,000	208,000
2	13,900	1,700	44,500	8,000	59,200 [c]	.... [d]	26,600	1,000	24,600	5,200	62,200 [c]	.... [d]	26,000	2,400	19,900	2,840
3	6,000	400	6,600	700	....	....	4,800	700	10,000	3,500	....	....	6,900	650	....	....
4	....	....	....	....	....	....	....	....	2,400	300	....	....	....	....	....	....
5	....	....	....	....	....	....	....	....	1,020	100	....	....	....	....	....	....
6	....	....	....	....	....	....	....	....	900	0	....	....	....	....	....	....
Total	1,369,900	66,100	1,291,100	31,000	1,206,200	40,000	1,122,900	342,200	1,528,920	93,800	1,391,200	65,400	1,338,800	88,750	1,311,900	210,840
% of dose	87.8	4.2	82.8	2.0	77.3	2.6	72.0	21.9	98.0	6.0	89.2	4.2	85.8	5.7	84.1	13.5
Organ																
Heart	25		55		0		49		25		13		45		26	
Liver	6,551		7,189		15,200		9,889		3,692		11,519		9,696		20,619	
Lung	63		47		81		70		47		66		60		76	
Kidney	354		126		0		82		39		60		87		268	
Spleen	0		15		19		8		12		6		10		11	
Testis	0		151		75		38		..		..		..		72	
Stomach	145		162		0		44		0		255		108		102	
Intestine	896		970		952		301		514		519		438		0	

[a] 1 mg. of ATA-5-C^{14} measured 1.56×10^6 c.p.m. [b] M = male, F = female; average body weight of male rat = 326 grams, of female rat = 214 grams. [c] From 24- to 68-hour period. [d] Not determined.

Table II. Percentage of Radioactivity from Alcohol Extracts of Rat Liver Samples from Rats Sacrificed at Various Times after Dosing

Time, Hours	ATA, %	Metabolite-1, %
0.5	100	0
1	95.4	4.6
2	80.4	19.6
3	46.2	53.8
4	36.4	63.6
6	45.5	54.5
12	42.3	57.7
18	34.5	65.5
24	0	100

Table III. Comparative Accumulation of C^{14} in Various Tissues of Male Rats Sacrificed after a Single Oral Dose of 1.0 Mg. of ATA-5-C^{14}

(Radioactivity was expressed as c.p.m. per 100 mg. of dry tissue)

Tissue or Organ	Time after Dosing, Hours										
	0.5 (No. 20) [a]	1 (No. 18)	2 (No. 21)	3 (No. 17)	4 (No. 22)	6 (No. 19)	12 (No. 38)	18 (No. 39)	24 (No. 23)	48 (No. 16)	72 (No. 15)
Blood	940	1,810	1,000	1,016	773	528	82	18	37	12	12
Brain	0	918	515	581	546	337	66	22	0	..	..
Fat	0	0	0	0	0	0	0	0	0	0	0
Heart	88	1,530	605	750	733	530	66	0	33	12	19
Intestine	168	475	636	440	408	295	602	235	49	0	6
Kidney	42	1,710	194	1,100	1,200	523	281	102	122	35	16
Liver	194	1,223	1,559	1,430	2,083	1,242	1,173	689	790	356	334
Lung	162	3,633	987	886	848	477	107	34	48	32	22
Muscle	21	989	345	437	527	283	46	7	32	13	0
Spleen	562	988	116	598	685	491	71	13	36	11	10
Skin	0	0	0	0	0	0	..	..	0	0	0
Stomach	43,050	916	18,010	1,987	2,288	2,070	1,142	128	108	14	10
Testis	46	827	408	844	1,270	690	112	45	55	14	7
Urogenital organ	64	857	142	704	728	1,231	71	25	24	..	..

[a] Average body weight of these male rats = 354 grams.

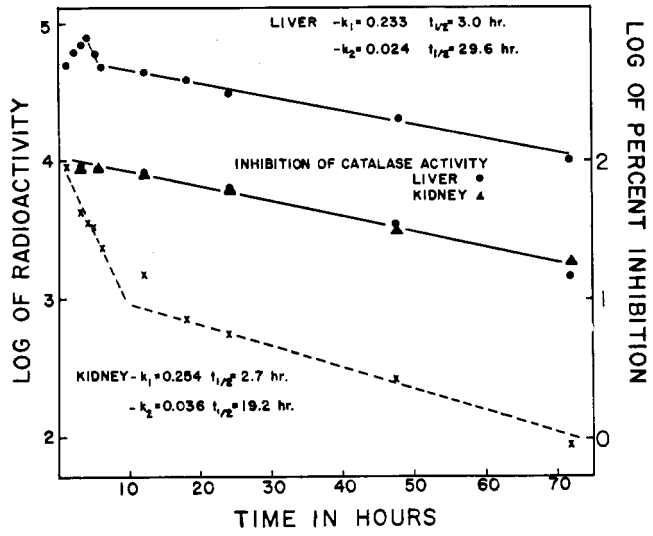

Figure 2. In vivo kinetics of C^{14} elimination in the liver and kidney of rats following a single oral dose of ATA-5-C^{14}

Data on the inhibition of catalase activity in rat liver and kidney were from Heim, Appleman, and Pyfrom (4)

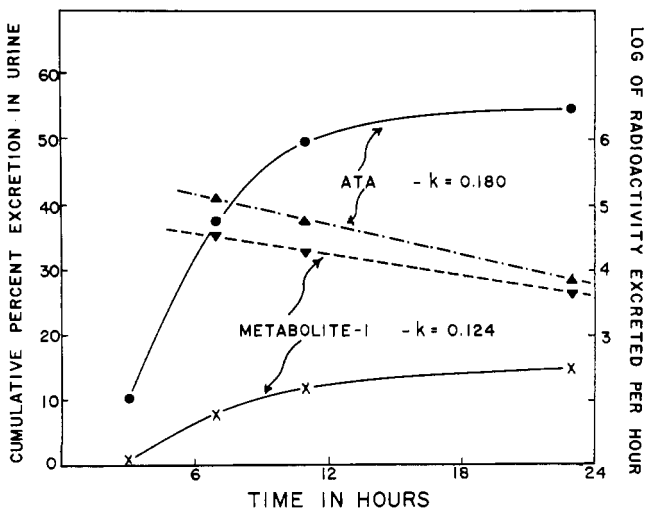

Figure 3. Average cumulative recoveries of ATA-5-C^{14} and metabolite-1 in urine and their rates of elimination

Table IV. Relative Composition of Radioactivity in Rat Urine after a Single Oral Dose of ATA-5-C^{14}

(Average of three rats)

Time after Dosing, Hours	ATA		Metabolite-1		Metabolite-2	
	C.P.M.	%	C.P.M.	%	C.P.M.	%
0–3	183,800	95	9,680	5	0	0
3–7	487,430	79	129,570	21	0	0
7–11	220,460	73	75,500	25	6,040	2
11–23	86,400	60	53,280	37	4,320	3

most cases, the abdominal muscle, skin, and fatty tissue showed no activity.

Table III shows the comparative concentration of ATA-5-C^{14} in various organs and tissues from male rats sacrificed at different times after dosing. In most vital organs, the maximum concentration was reached 1 hour after oral administration of ATA, and then remained approximately at the same level for the next 2 to 3 hours. After that, the radioactivity in most organs started to decrease and, with the exception of liver, reached a virtually zero level in 3 days. When the average values obtained from animals sacrificed between 1 to 6 hours were used, the highest concentration was found in the liver and was nearly 50% greater than the ATA in the blood. The next highest was found in the kidney and was closest to the level in the blood. The activities in other tissues, generally 20 to 50% less than the level found in the blood, were in decreasing order as follows: urogenital organ, spleen, testis, lung, heart, brain, and abdominal muscle. The skin and fatty tissue were not radioactive. The unusually high concentration found in the lung of rat No. 18 was probably due to a small amount of the solution that may have accidentally entered into the lung during administration. This also resulted in a higher concentration in the blood, heart, spleen, and muscle, and in a low value in the stomach. The entering of ATA activity into the brain was somewhat slower than the other internal organs, since there was no radioactivity in the brain sample from the animal sacrificed 30 minutes after dosing.

Kinetic analyses from the radioactivity accumulation data in various organs reveal that the rate constant of ATA elimination, k, in most organs is from -0.181 to -0.328 (Figure 1). The time required for the removal of half the ATA concentration is from 2.1 hours in the spleen to 3.8 hours in the testis. The extremely short range of the rate constant, k_1, in the liver, is possibly due to the rapid formation of metabolite-1 with a limited new supply of ATA after the fourth hour. In addition to the first rate constant found in the liver and kidney, a second rate constant, k_2, is evident, and the $t_{1/2}$ is 29.6 and 19.2 hours for the liver and kidney, respectively. These plots are shown in Figure 2.

Paper chromatographic and radioautographic results of alcohol extracts of internal organs from male rat No. 21 indicated the presence of a radioactive spot in addition to radioactive ATA. Only liver and kidney contained significant amounts of this metabolite (53.8 and 35.6%, respectively), and the other organs contained only a trace (less than 5%). Since no free ATA was found in the alcohol extract of the rat liver 24 hours after dosing, the second rate constant for C^{14} elimination noted in both the liver and kidney was probably for the elimination of this radioactive metabolite.

Table IV presents the average values of ATA and C^{14}-metabolite elimination in urine from three rats during the first 23 hours after dosing. Beginning in the urine sample from the 7- to 11-hour period, a small, second radioactive metabolite (R_F 0.19) was detected. This spot (metabolite-2) amounted to only 1 to 2% of the total urine activity. During the first 3 hours, the rats excreted 4.2% of the dose per hour. In the 3- to 7-hour period, the excretion rate increased to 9.9% of the dose per hour, then decreased to 4.8% per hour between the 7- to 11-hour period, and to 0.8% per hour in the last 12-hour period. Total urinary excretion rates for ATA residue and its metabolites are shown in Figure 3.

The radioactivity in the alcohol extract of feces also was shown to contain ATA residue and metabolite-1. The percentage distribution between radioactive ATA and its metabolite from the alcohol extract of the first-day feces sample was generally similar to the result found in the first-day urine sample from the same animal. This observation suggested that the activity found in the feces may be in part a contamination from the urine.

Discussion

The rapid loss of radioactivity from the stomach following the oral administration of ATA-5-C^{14} suggests that this chemical may enter the blood stream

primarily by way of the gastric mucosa. The ATA molecule is generally completely absorbed in the gastrointestinal tract. The accumulation data indicate that after absorption ATA is distributed through most body tissues. Highest radioactivity in all tissues is generally reached within 1 hour after dosing, maintaining the same level for 2 to 3 hours before the activity starts to decrease. The rate of elimination of ATA from all tissues is fast and requires only from 2 to 3 hours for the removal of one half of its concentration. ATA-5-C^{14} is not completely oxidized by rats, and the C^{14} does not appear in the breath. Elimination of this chemical from the body is chiefly from the kidney by way of urine excretion.

Liver is the site of ATA metabolism with the formation of a radioactive metabolite. Removal of this metabolite from the liver is much slower than ATA. In view of the similarity between the rate of elimination of this metabolite from liver or kidney and the rate of decrease on catalase inhibition as reported by Heim, Appleman, and Pyfrom (*4*) in the same organs, the ATA metabolite might be the responsible toxic agent. Also, no free ATA is present in the liver sample after 24 hours following dosing, yet the inhibition of catalase activity still persists. On the other hand, the concentration of ATA in blood is reasonably high (next only to the concentration found in the liver), while blood catalase activity of the treated animals remained normal. However, Tephly *et al.* (*10*) concluded from their experiment that the failure of ATA to inhibit blood catalase is due to an insufficient amount of hydrogen peroxide in the erythrocytes. Price, Rechcigl, and Hartley (*8*) demonstrated that the inhibition of liver catalase activity by ATA is a result of catalase destruction. The rate constant, k_D, for catalase destruction is 0.027 which is reasonably close to the result for the removal of metabolite-1 from liver ($k_2 = -0.024$). Isolation and identification of metabolite-1 are now in progress. Whether or not this metabolite-1 is identical to ATA-catalase complex as reported by others (*5–7*) remains unanswered.

Acknowledgment

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ENHANCEMENT OF HERBICIDE ACTIVITY

Relation of Structure of Ethylene Oxide Ether-Type Nonionic Surfactants to Herbicidal Activity of Water-Soluble Herbicides

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SURFACTANTS have had a considerable impact on the development and use of herbicides in recent years. In agricultural applications, some of the potential of surfactants for lowering costs of weed control, increasing the efficiency of herbicides, and reducing residue hazards is already being realized. However, a fundamental technology of surfactant-herbicide systems is needed to promote further industrial and agriculture applications.

Previous reports demonstrated that toxicity of herbicides to intact plants could be greatly enhanced by some surfactants, unaffected by others, or significantly suppressed by a third group (*4, 5*). The effects of a surfactant were related to its concentration but not to its ionogenic class. Phytotoxicities of surfactants themselves to two species (*2*) and toxicity of one herbicide to crabgrass (*5*) were influenced by structure of surfactants.

Nonionic surfactants derive their hydrophilic characteristics from nonionizable groups, such as phenolic and alcoholic hydroxyls, carbonyl oxygens of esters and amides, ether oxygens, and analogous sulfur-containing configurations. Water solubility is a function of the number of hydrophilic configurations in the molecule. Nonionic surfactants are little influenced by the degree of water hardness and are compatible with most organic ions, characteristics which account for their widespread use in pesticide formulations.

Because of their low production cost, the ethylene oxide adducts of long-chain alcohols and phenols (the ether-linked nonionics) are probably more frequently encountered as emulsifiers and wetting agents than any other type of nonionic surfactants. Ethylene oxide can be added to the hydrophobic moieties in an essentially step-wise fashion, but the reaction products represent a range of adducted species. The average mole ratio of ethylene oxide (EO) per hydrophobe is used to characterize the mixture (*6*).

Objectives of the current investigations were to evaluate critically the relations of hydrophile-lipophile structural configurations of related surfactants to their effects on herbicidal activity and to study the correlation of these relations with the physical-chemical properties of the spray solutions. This paper characterizes some of the activity-structural relations in the ether-linked polyoxyethylene nonionic surfactants and uses the EO mole ratio as an experimental variable.

Materials and Methods

Thirty-four nonionic surfactants (Table I), representing ether-ethoxylates of 15 hydrophobes belonging to four major hydrophobe groups, were selected