sulfonamides, active investigation is continuing in these laboratories.

ACKNOWLEDGMENT

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Behavior and Fate of Two Phenylpyridazinone Herbicides in Cotton, Corn, and Soybean

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The absorption, translocation, and metabolism of [14C]SAN 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone] and [14C]SAN 9789 (norflurazone) [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone] by cotton (*Gossypium hirsutum* L. "Coker 203"), corn (*Zea mays* L. "WF 9"), and soybean [*Glycine max* (L.) Merr. "Lee"] were investigated. Differences in absorption and translocation appeared to be major factors determining the tolerance of plants to SAN 6706 and SAN 9789. Autoradiographic and combustion analyses indicated that most of the radioactivity absorbed by cotton (tolerant) was retained in the roots while significantly more radioactivity was translocated into the shoots of soybean (susceptible) and corn (very susceptible). Tolerant cotton mediated only limited breakdown of the herbicides while the susceptible species rapidly degraded them by the process of N-demethylation. The rapid degradation of SAN 6706 by corn and soybean did not represent a detoxification mechanism since the primary degradation product formed (*i.e.*, SAN 9789) was more phytotoxic than SAN 6706.

SAN 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone] and SAN 9789 [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone] are experimental herbicides which show promise for the selective preemergence control of many broadleaf and grassy weeds, including nutsedge (*Cyperus sp.*), in cotton fields.

The limited information available on the absorption and translocation of SAN 6706 and SAN 9789 indicated that the herbicides are absorbed by the roots of both susceptible and resistant species (Hilton *et al.*, 1969; Strang and Rogers, 1972, 1973). Hilton *et al.* (1969) reported that SAN 6706 was resistant to metabolic detoxication in plants. Strang and Rogers (1972, 1973) reported that SAN 6706 and SAN 9789 were readily degraded by several plant species. Soil degradation of SAN 6706 and SAN 9789 has since been reported (Rahn and Zimdahl, 1973; Rogers, 1972).

This present investigation was divided into three major parts. A study was made of the absorption, translocation, and metabolism of SAN 6706 by the three plant species. A similar study was conducted with SAN 9789. Finally, the absorption and translocation of SAN 6706 was compared to that of SAN 9789 within each plant species. The latter study was initiated to determine whether differences in the movement of the two herbicides in the test species that were observed in the earlier experiments were statistically significant. To avoid excessive duplication of data, only the absorption and translocation results from the latter experiment were presented. These studies were conducted to determine if differential absorption, translocation, and/or metabolism could explain differences in the tolerance of cotton (resistant), corn (very susceptible), and soybean (susceptible) to SAN 6706 and SAN 9789. Such information should contribute to the intelligent utilization of these herbicides in the field.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L. "Coker 203"), corn (*Zea mays* L. "WF 9"), and soybean [*Glycine max* (L.) Merr. "Lee"] seed were germinated in flats of vermiculite, which were watered as required with half-strength Hoagland and Arnon's No. 2 nutrient solution (Hoagland and Arnon, 1950). Seven ten-day-old seedlings were transferred to half-strength nutrient solution for a 3-day equilibration period. Uniform plants were selected and transferred to

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1-pint plastic pots containing 300 ml of aerated half-strength nutrient solution which contained a known concentration of the ^{14}C-labeled herbicide. The plastic pots were painted on the outside to exclude light from the plant roots. The experiments were conducted in a growth chamber programmed for a 14-hr day at 30° and a 10-hr night at 25°. The light intensity at plant level was approximately 21,520 lx from a mixture of fluorescent and incandescent lamps.

The herbicide in the SAN 6706 experiment was applied *via* the nutrient solution at a rate of 1.0 ppmw. In the SAN 9789 study and in the SAN 6706 *vs.* SAN 9789 comparison study, the herbicides were applied at 0.5 ppmw. The specific activity of the ^{14}C-labeled herbicides was 9.46 μCi/mg for SAN 6706 and 11.43 μCi/mg for SAN 9789. Both herbicides were labeled in the four and five positions of the pyridazinone ring.

Treatment times of 24, 48, and 96 hr were employed in all experiments. An 8-day treatment period for cotton was included in the SAN 6706 *vs.* SAN 9789 comparison study. The selection of the treatment times was determined largely by the sensitivity of corn and soybean to the herbicides. Preliminary tests demonstrated that corn seedlings were generally killed after 4–6 days exposure to a 1.0 ppmw SAN 6706 solution. Soybean seedlings lived for 5–7 days at this concentration, while cotton plants tolerated this level of SAN 6706 for up to 3 weeks before exhibiting severe toxicity symptoms. There were at least three replications per treatment with each replication consisting of four plants. Each experiment was repeated at least twice.

Absorption. The amount of herbicide absorbed by each plant species was determined by measuring the loss of radioactivity from the treatment solutions. After treatment, the plants were removed from the herbicide solutions, their roots washed in 300 ml of distilled water, rinsed in running tap water, and blotted dry, and the fresh weights were recorded. The volume of the remaining treatment solution and the root-wash water was measured and 100-μl samples were radioassayed using a Beckman LS-250 liquid scintillation system. The quantity of herbicide removed from the treatment solution was calculated and the absorption data expressed as micrograms of herbicide absorbed per gram of fresh plant tissue. This form of data expression was selected in order to present the SAN 6706 and SAN 9789 data on a uniform basis. Since the compounds differed in their specific activities, a disintegrations per minute per gram basis would not be valid.

Translocation. The translocation patterns of the herbicide absorbed by each plant species were determined by making gross autoradiographs of one or more plants from each replicate, using the techniques of Crafts and Yamaguchi (1964).

Localization of the absorbed herbicides within the plants was determined quantitatively by combusting 50-mg (dry weight) samples of the freeze-dried plants used to obtain autoradiographs. The plants were separated into root and shoot segments and ground in a Wiley mill to pass a 40-mesh screen. Weighed samples were wrapped in black, ashless paper and ignited using standard oxygen flash combustion techniques (Davidson *et al.*, 1970). An 18-ml volume of a combination CO_2-trapping solution and scintillation cocktail, consisting of phenethylamine (270 ml), methanol (270 ml), toluene (460 ml), PPO (5 g), and POPOP (100 mg) per liter, was injected into each combustion flask. After a 30-min condensing period in an ice–water bath, a 15-ml aliquot of the trapping solution was radioassayed.

The radioactivity present in each sample was calculated and the data were expressed as micrograms of herbicide per gram (dry weight) of plant tissue, and also on a percentage basis of the total radioactivity present in roots *vs.*

shoots. In making these calculations it was assumed that all of the radioactivity detected was the intact herbicide.

Metabolism. The metabolism of [^{14}C]SAN 6706 and [^{14}C]SAN 9789 by the three plant species was determined by homogenizing three plants from each replication with 100 ml of acetonitrile for 15 min in a high speed Virtis blender. The homogenates were suction filtered twice, using 75-ml volumes of acetonitrile. These high-speed, long-duration extractions were found to be essential for obtaining a high extraction efficiency. The filtrates were combined and concentrated to 25 ml. Samples of 100 μl were radioassayed to determine the total radioactivity extracted, and thus permit the determination of the efficiency of the extraction procedure. The filtrates were further concentrated to a 2-ml volume and known aliquots (50 or 100 μl) were spotted on 20 × 20 cm glass thin-layer chromatography plates coated with 200 μ of silica gel G. All of the time treatments for a given replication of a given plant species were spotted on a single plate. In addition to the extracts from the 24, 48, and 96 hr herbicide treatments, the extract from a check (untreated plants), a check which was spiked with the intact labeled herbicide at the initiation of the extraction procedure, and a combined standard which contained synthesized possible metabolites, were spotted on each tlc plate. The spiked check served to determine whether any degradation of the intact herbicide occurred during the extraction procedure, while the synthesized possible metabolites served as reference standards in the tentative identification of degradation products.

Duplicate plates were developed in benzene–ethanol (9:1, v/v) and chloroform–ethanol (95:5, v/v) solvent systems. These two solvent systems were selected from several tested in preliminary experiments, on the basis of their providing good separation and resolution of the various probable metabolites. The tlc plates were developed to a height of 15 cm, air-dried, and then viewed in a Chromato-Vue Cabinet equipped with short-wave (2537 Å) and long-wave (3650 Å) ultraviolet lamps to determine the locations of the reference compounds. The plates were then exposed to Kodak No-Screen X-ray film for 4 weeks. The positions of the intact ^{14}C-labeled herbicides and their ^{14}C-labeled metabolites were located on the plates by comparing the chromatograms with the corresponding autoradiographs. The ^{14}C-labeled areas on the tlc plates were transferred to vials and radioassayed. The relative quantities of radioactivity present in the various fractions were expressed as percentages of the total radioactivity present on a given chromatogram.

RESULTS AND DISCUSSION

Absorption. The absorption data indicate that both SAN 6706 and SAN 9789 were readily absorbed by all three plant species, and that the amount absorbed increased significantly with increasing treatment time (Figure 1, Table I). Soybean absorbed the greatest amount of the herbicides, followed by cotton and corn, respectively. The observed differences in absorption between soybean (susceptible) and cotton (tolerant) appear to account at least partially for the greater sensitivity of soybean to SAN 6706 and SAN 9789. However, other factors must be important since corn, the most susceptible species, absorbed the least amount of the herbicides.

The results also indicate that both cotton and soybean absorbed significantly greater quantities of SAN 6706 than SAN 9789, at all treatment times. In corn there was no significant difference between the amounts of SAN 6706 and SAN 9789 absorbed, although the uptake of SAN 6706 appeared to be slightly greater at each treatment time.

Translocation. The autoradiographs in Figures 1A, 1B, and 1C show the distribution of radioactivity in corn, soy-
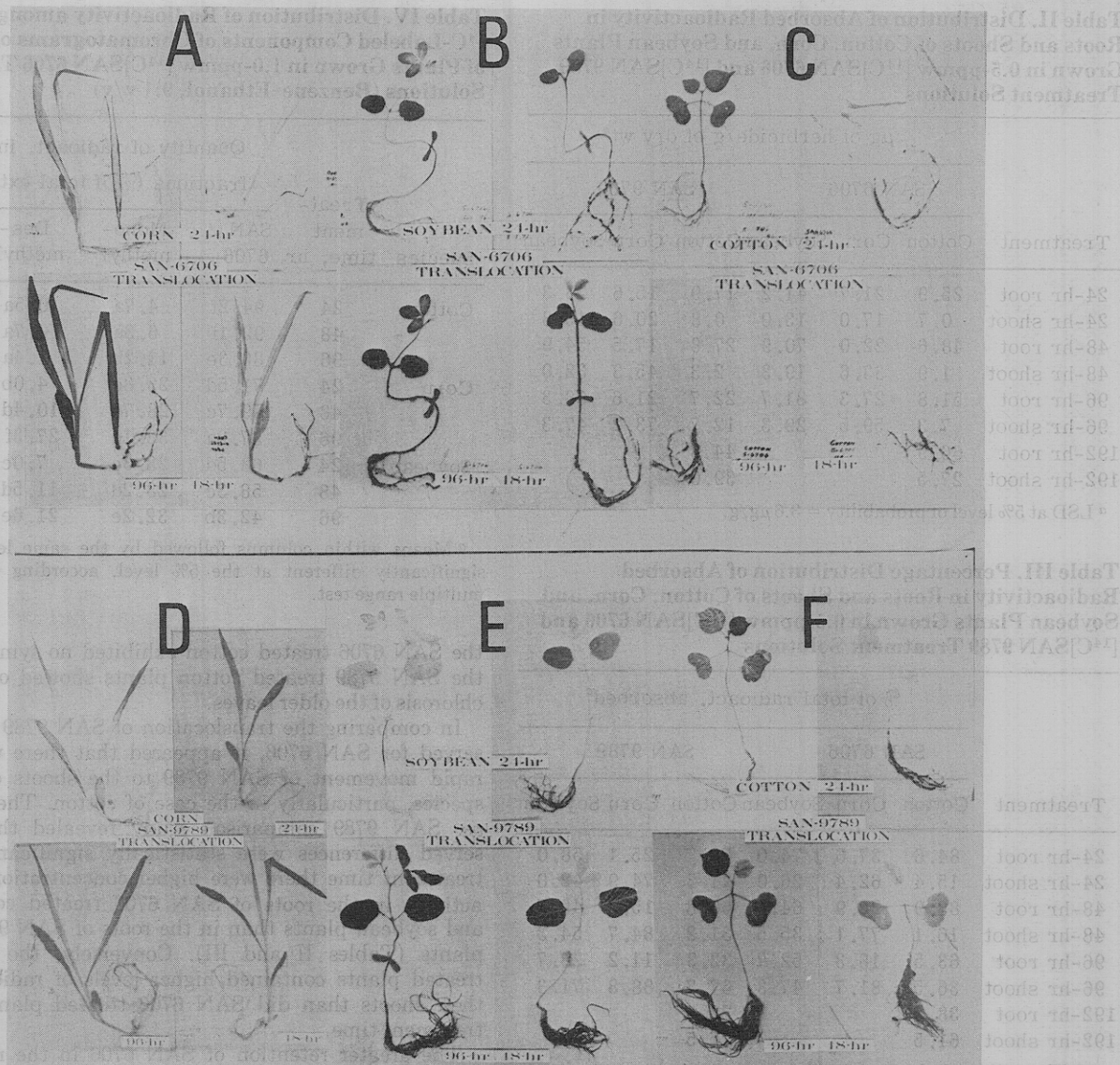
Figure 1. Autoradiographs of corn, soybean, and cotton plants grown in [^{14}C]SAN 6706 and [^{14}C]SAN 9789 treatment solutions for 24, 48, and 96 hr. Plant specimens used for the 96-hr autoradiographs are shown in the upper left-hand corner of each figure: (A–C) [^{14}C]SAN 6706 translocation in corn, soybean, and cotton, respectively; (D–F) [^{14}C]SAN 9789 translocation in corn, soybean, and cotton, respectively.

bean, and cotton plants, respectively, grown in SAN 6706 treatment solutions for 24, 48, and 96 hr. Figures 1D, 1E, and 1F show the corresponding data for SAN 9789 treated plants. In each figure the intact plant used to prepare the 96-hr autoradiograph is shown in the upper left-hand frame for comparative purposes.

Table I. Absorption of [^{14}C]SAN 6706 and [^{14}C]SAN 9789 from 0.5-ppmw Treatment Solutions by Cotton, Corn, and Soybean

Plant species	Herbicide	μg of herbicide absorbed/g fresh wt[a] at treatment time			
		24 hr	48 hr	96 hr	192 hr
Cotton	SAN 6706	4.05	7.25	9.20	8.35
	SAN 9789	2.63	4.83	5.80	6.95
Corn	SAN 6706	1.63	4.00	6.47	
	SAN 9789	1.23	3.93	6.37	
Soybean	SAN 6706	4.03	8.33	15.50	
	SAN 9789	2.67	6.90	11.87	

[a] LSD at 5% level of probability = 0.9 μg/g.

The autoradiographic data indicate that there are very marked differences in the translocation patterns of SAN 6706 in cotton (Figure 1C) as compared to corn (Figure 1A) and soybean (Figure 1B). At all treatment times the vast majority of the absorbed herbicide was retained in the roots of the cotton plant. In contrast, the data for corn and soybean showed that in these susceptible species there was a rapid and significantly greater translocation of much of the absorbed radioactivity into the shoots. Similar distribution patterns were observed for SAN 9789, with the exception that greater quantities of radioactivity appeared to be present in the shoots of all three species. This difference was most noticeable in the cotton plants (Figure 1F). However, a large fraction of the radioactivity present in the cotton shoots appeared to be concentrated in the lysigenous or pigment glands of the stem and leaves. Any herbicide accumulating in these glands would be prevented from reaching the photosynthetic areas of the leaves, where the pyridazinone herbicides exert their phytotoxic action on the plant (Hilton et al., 1969).

The plant material used in the autoradiographic studies was combusted and radioassayed to obtain quantitative distribution data for SAN 6706 and SAN 9789. The results are presented in two forms, i.e., on a microgram of herbi-

Table II. Distribution of Absorbed Radioactivity in Roots and Shoots of Cotton, Corn, and Soybean Plants Grown in 0.5-ppmw [14C]SAN 6706 and [14C]SAN 9789 Treatment Solutions

	μg of herbicide/g of dry wt[a]					
	SAN 6706			SAN 9789		
Treatment	Cotton	Corn	Soybean	Cotton	Corn	Soybean
24-hr root	25.9	21.7	41.2	17.9	15.6	26.3
24-hr shoot	0.7	17.0	13.0	0.8	20.6	15.3
48-hr root	48.6	22.0	70.9	27.8	17.5	54.9
48-hr shoot	1.9	33.6	19.3	2.3	45.3	32.0
96-hr root	51.8	27.3	81.7	22.7	21.6	68.3
96-hr shoot	7.3	59.5	29.3	12.7	73.2	47.3
192-hr root	66.9			44.8		
192-hr shoot	27.5			39.0		

[a] LSD at 5% level of probability = 3.6 μg/g.

Table III. Percentage Distribution of Absorbed Radioactivity in Roots and Shoots of Cotton, Corn, and Soybean Plants Grown in 0.5-ppmw [14C]SAN 6706 and [14C]SAN 9789 Treatment Solutions

	% of total radioact. absorbed[a]					
	SAN 6706			SAN 9789		
Treatment	Cotton	Corn	Soybean	Cotton	Corn	Soybean
24-hr root	84.6	37.6	74.0	78.7	25.1	58.0
24-hr shoot	15.4	62.4	26.0	21.3	74.9	42.0
48-hr root	83.9	22.9	64.5	68.8	15.3	45.7
48-hr shoot	16.1	77.1	35.5	31.2	84.7	54.3
96-hr root	63.5	18.3	52.7	32.3	11.2	28.7
96-hr shoot	36.5	81.7	47.3	67.7	88.8	71.3
192-hr root	38.5			22.5		
192-hr shoot	61.5			77.5		

[a] LSD at 5% level of probability = 5.8 μg/g.

cide per gram of dry plant tissue basis (Table II), and as a percentage distribution of the total absorbed radioactivity in roots and shoots (Table III). These data substantiate the autoradiographic results, in that at all treatment times the shoots of the susceptible corn and soybean plants contained significantly greater concentrations of radioactivity than did shoots of cotton plants (tolerant). The data, expressed on a percentage basis, indicate a far more efficient translocation of absorbed radioactivity into the shoots of the susceptible species.

SAN 6706 and SAN 9789 are preemergence herbicides which act by inhibiting both the photosynthetic process and the development of chloroplasts and the photosynthetic pigments (Bartels and Hyde, 1970; Hilton et al., 1969, 1971). To be active, the chemicals must be absorbed by the roots of plants and translocated to the leaves. Thus the absorption and translocation of the pyridazinone herbicides are obviously major factors in a consideration of their phytotoxicity. The observed differences in the absorption and, to a greater extent, translocation of SAN 6706 and SAN 9789 in tolerant cotton as compared to susceptible corn and soybean, appear significant enough to adequately explain the established differences in the susceptibility of these species to the herbicides. These differences were reflected in the appearance of toxicity symptoms in corn and soybeans as early as 24 hr after exposure to the herbicide solutions. After 96-hr exposure, both corn and soybean showed extensive chlorosis and necrosis while

Table IV. Distribution of Radioactivity among 14C-Labeled Components of Chromatograms of Extracts of Plants Grown in 1.0-ppmw [14C]SAN 6706 Treatment Solutions (Benzene-Ethanol, 9:1 v/v)

		Quantity of radioact. in indicated fractions (% of total extracted)[a]			
Species	Treatment time, hr	SAN 6706	Monomethyl	Desmethyl	Unidentified
Cotton	24	94.2f	4.7a	0.5a	0.6a
	48	92.1f	6.3a	0.7a	0.9ab
	96	86.3e	11.2b	1.4a	1.1ab
Corn	24	71.5d	23.8c	4.0b	0.7ab
	48	59.7c	28.7d	10.4d	1.2ab
	96	37.2a	33.1e	27.3f	2.5c
Soybean	24	68.5d	23.3c	7.0c	1.2a
	48	58.3c	28.2d	11.5d	2.0bc
	96	42.3b	32.2e	21.6e	3.9d

[a] Means within columns followed by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

the SAN 6706 treated cotton exhibited no symptoms, and the SAN 9789 treated cotton plants showed only limited chlorosis of the older leaves.

In comparing the translocation of SAN 9789 to that observed for SAN 6706, it appeared that there was a more rapid movement of SAN 9789 to the shoots of all three species, particularly in the case of cotton. The SAN 6706 vs. SAN 9789 comparison study revealed that the observed differences were statistically significant. At each treatment time there were higher concentrations of radioactivity in the roots of SAN 6706 treated cotton, corn, and soybean plants than in the roots of SAN 9789 treated plants (Tables II and III). Conversely, the SAN 9789 treated plants contained higher levels of radioactivity in their shoots than did SAN 6706 treated plants, at each treatment time.

The greater retention of SAN 6706 in the roots of the test species and the more rapid translocation of SAN 9789 into their shoots is probably a function of the greater water solubility of SAN 9789 (40 ppm) as compared to SAN 6706 (10.5 ppm at 25°). The pyridazinone herbicides are reported to be transported primarily in the xylem (Frank and Switzer, 1969; Stephenson and Ries, 1967). The fourfold greater water solubility of SAN 9789 over SAN 6706 should result in a more rapid translocation of SAN 9789 to the shoots of treated plants, via the transpiration stream.

The more rapid accumulation of toxic levels of SAN 9789 in the shoots of cotton, corn, and soybean resulted in the earlier appearance of toxicity symptoms in these plants than in SAN 6706 treated plants. The difference was most apparent in cotton. After 192 hr in the SAN 6706 treatment solution, cotton exhibited only limited chlorosis, primarily along the leaf midrib and other large veins. In contrast, the SAN 9789 treated plants exhibited acute toxicity symptoms, including extensive chlorosis, necrosis, and wilting of the older leaves. Necrosis occurred earliest in the leaf tissues surrounding the lysigenous glands, apparently due to the concentration of the herbicides in these regions, as was indicated by the autoradiographic data.

The lesser tolerance of cotton to SAN 9789 as compared to SAN 6706 may result in a lower margin of safety in field applications of SAN 9789. However, since the data suggest that it is generally more phytotoxic than SAN 6706, it is likely that SAN 9789 can be applied at lower rates of application than those required with SAN 6706.
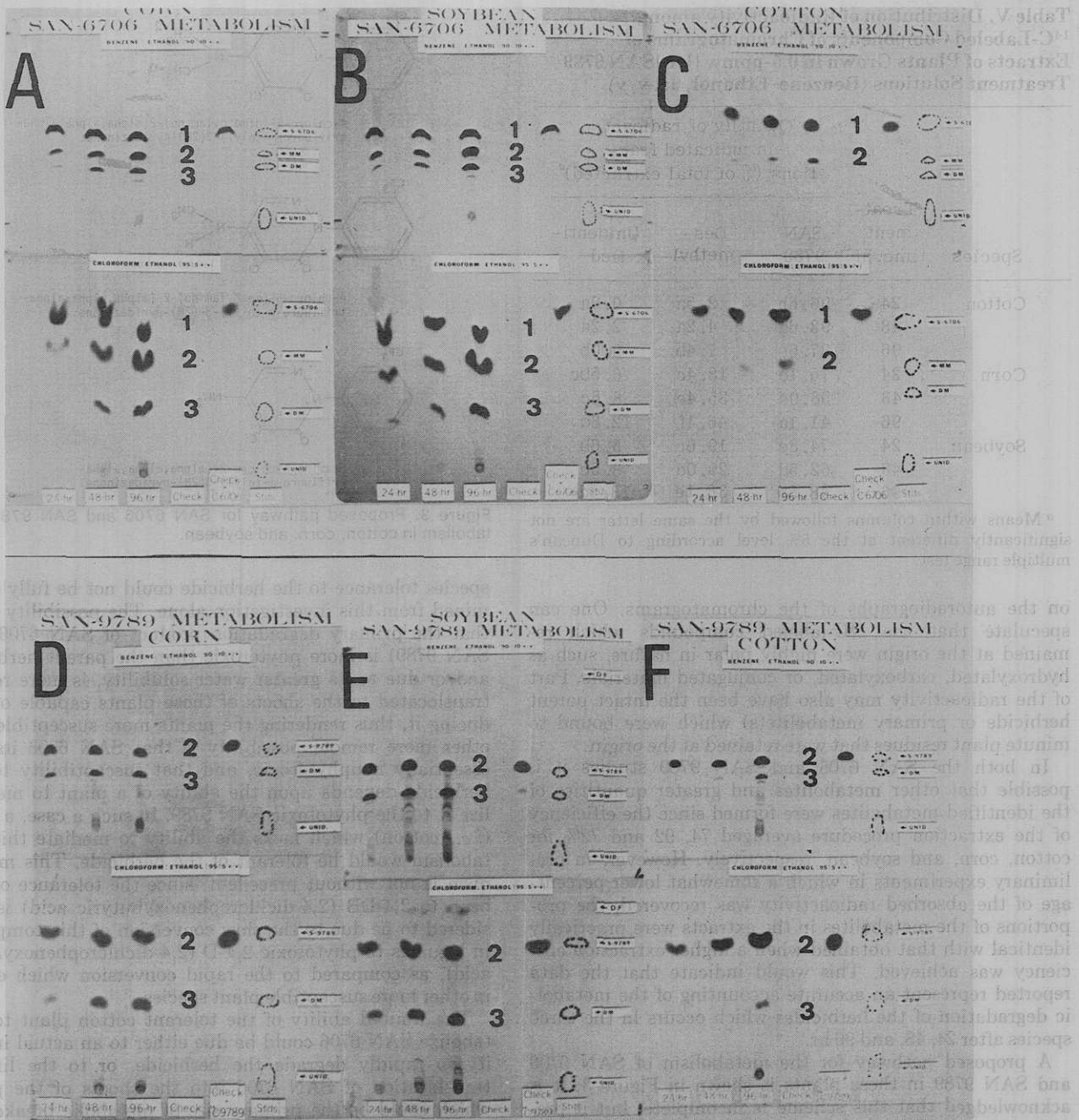
Figure 2. Autoradiographs of thin-layer chromatograms of standards or known compounds and of extracts of corn, soybean, and cotton plants treated with [^{14}C]SAN 6706 or [^{14}C]SAN 9789 for 24, 48, and 96 hr: (A–C) [^{14}C]SAN 6706 corn, soybean, and cotton, respectively; (upper) benzene–ethanol (9:1, v/v); (lower) chloroform–ethanol (95:5, v/v); (D–F) [^{14}C]SAN 9789 corn, soybean, and cotton, respectively; (upper) benzene–ethanol (9:1, v/v); (lower) chloroform–ethanol (95:5, v/v). Probable identification of major ^{14}C-labeled components as follows: (1) SAN 6706; (2) SAN 9789 (monomethyl derivative); and (3) SAN 9789 (demethylated derivative).

Metabolism. Differences in the ability of resistant *vs.* susceptible species to absorb and translocate SAN 6706 and SAN 9789 appeared to be great enough to account for the differences in their susceptibility to the herbicides. However, metabolic studies were conducted to determine if differential degradation of the herbicides by cotton, corn, and soybean was also involved in determining species sensitivity.

The data indicate that little SAN 6706 degradation occurred in the cotton plant (Table IV, Figure 2C). After 96 hr, greater than 86% of the total ^{14}C extracted chromatographed with the intact parent herbicide. Only limited amounts of the monomethyl derivative and trace amounts of the desmethyl derivative were identified. In contrast, both corn and soybean readily degraded SAN 6706 (Table

IV, Figures 2A, 2B). Significant amounts of the monomethyl and desmethyl derivatives were observed after only 24 hr. In these susceptible species the per cent of ^{14}C present in these metabolite fractions increased rapidly with increasing treatment time, while that in the intact herbicide fraction decreased correspondingly. No significant degradation beyond the desmethyl derivative was observed, although after 96 hr there were indications of trace amounts of other ^{14}C-labeled metabolites.

The SAN 9789 metabolism data indicated that the degradation of this herbicide also occurred far more rapidly in corn and soybean than it did in cotton (Table V, Figures 2D, 2E, 2F). The major degradative product in all species was the desmethyl derivative. Trace amounts of several unidentified ^{14}C-labeled compound(s) are also apparent

Table V. Distribution of Radioactivity among ¹⁴C-Labeled Components of Chromatograms of Extracts of Plants Grown in 0.5-ppmw [¹⁴C]SAN 9789 Treatment Solutions (Benzene–Ethanol, 9:1 v/v)

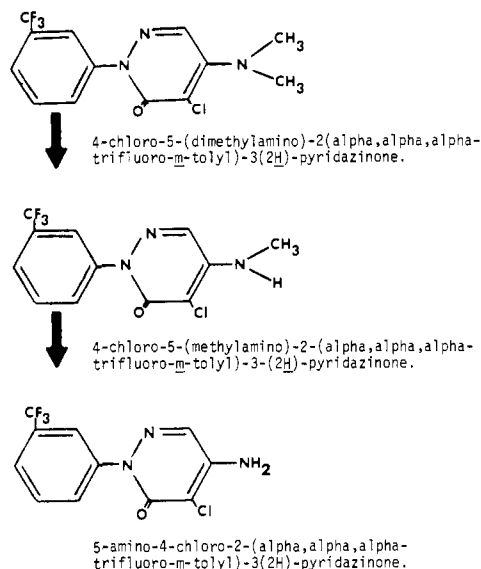| Species | Treatment time, hr | Quantity of radioact. in indicated fractions (% of total extracted)[a] | | |
		SAN 9789	Des-methyl	Unidentified
Cotton	24	96.6h	2.5a	0.9a
	48	93.6g	4.2a	2.2a
	96	87.6f	7.4b	5.0b
Corn	24	75.1e	18.4c	6.5bc
	48	56.0c	35.4e	8.6c
	96	41.1a	46.1f	12.8d
Soybean	24	74.8e	19.6c	5.6b
	48	62.5d	29.0d	8.5c
	96	48.5b	38.1e	13.5d

[a] Means within columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Figure 3. Proposed pathway for SAN 6706 and SAN 9789 metabolism in cotton, corn, and soybean.

on the autoradiographs of the chromatograms. One can speculate that the ¹⁴C-labeled compounds which remained at the origin were highly polar in nature, such as hydroxylated, carboxylated, or conjugated materials. Part of the radioactivity may also have been the intact parent herbicide or primary metabolite(s) which were bound to minute plant residues that were retained at the origin.

In both the SAN 6706 and SAN 9789 studies it is possible that other metabolites and greater quantities of the identified metabolites were formed since the efficiency of the extraction procedure averaged 74, 92 and 72% for cotton, corn, and soybean, respectively. However, in preliminary experiments in which a somewhat lower percentage of the absorbed radioactivity was recovered, the proportions of the metabolites in the extracts were practically identical with that obtained when a higher extraction efficiency was achieved. This would indicate that the data reported represent an accurate accounting of the metabolic degradation of the herbicides which occurs in the three species after 24, 48, and 96 hr.

A proposed pathway for the metabolism of SAN 6706 and SAN 9789 in these plants is shown in Figure 3. It is acknowledged that this scheme is incomplete, but it presents the information concerning the degradation of these herbicides which was obtained from these studies. As indicated, the degradation of SAN 6706 and SAN 9789 in all three species primarily involved N-demethylation. Such a mechanism is reported to be involved in the biological degradation of some carbamate, s-triazine, and substituted phenylurea herbicides (Kaufman, 1967; Shimabukuro, 1967; Williams, 1959). Smith and Sheets (1967) reported that the cleavage of one or both N-methyl groups from monuron [3-(p-chlorophenyl)-1,1-dimethylurea] or diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] resulted in a loss of herbicidal activity. However, the degradation of SAN 6706 by susceptible corn and soybean species does not represent a detoxification mechanism, as would be the case with many other herbicides. The loss of one N-methyl group from SAN 6706 results in the formation of the more phytotoxic monomethyl derivative, SAN 9789, which was the major metabolite found in the susceptible species at all treatment times. The demethylated metabolite is reported to be less phytotoxic than SAN 6706 (Hilton et al., 1969).

The importance of the differential metabolism of SAN 6706 by cotton, corn, and soybean in determining the

species tolerance to the herbicide could not be fully determined from this investigation alone. The possibility exists that the primary degradation product of SAN 6706 (i.e., SAN 9789) is more phytotoxic than the parent herbicide, and/or due to its greater water solubility, is more readily translocated to the shoots of those plants capable of producing it, thus rendering the plants more susceptible. Another more remote possibility is that SAN 6706 itself is essentially nonphytotoxic, and that susceptibility to this herbicide depends upon the ability of a plant to metabolize it to the phytotoxic SAN 9789. In such a case, a plant (i.e., cotton) which lacks the ability to mediate this metabolism would be tolerant of the herbicide. This mechanism is not without precedent, since the tolerance of soybean to 2,4-DB (2,4-dichlorophenoxybutyric acid) is considered to be due to the slow conversion of this compound in legumes to phytotoxic 2,4-D (2,4-dichlorophenoxyacetic acid), as compared to the rapid conversion which occurs in other more susceptible plant species.

The limited ability of the tolerant cotton plant to metabolize SAN 6706 could be due either to an actual inability to rapidly degrade the herbicide, or to the limited translocation of SAN 6706 into the shoots of the plant, which could be the primary site of metabolic breakdown. However, the limited degradation of SAN 9789 appeared to be due to the inability of cotton to rapidly metabolize the herbicide, since significant quantities of SAN 9789 were translocated to the shoots. This would support the possibility that cotton also lacks an active enzyme system for the degradation of SAN 6706, although some workers have reported that the successive cleavage of N-methyl groups, either in the same herbicide or in different compounds, may require different enzyme systems (Geissbuhler et al., 1963).

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Effect of Additives on the Formation of Nitrosamines in Meat Curing Mixtures Containing Spices and Nitrite

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Studies were carried out to determine the effect of an alkaline buffer and sodium ascorbate on the formation of N-nitrosamines in curing mixtures containing spices and nitrite. Four samples of such premixes prepared with or without the buffer were analyzed for nitrosamines after storage for 4–15 weeks at two different temperatures. The buffering (at pH 7.5–8.2) markedly reduced the formation of nitrosamines but did not prevent it completely. The formation of appreciable levels (up to 4 ppm) of nitrosamines was observed in most of the nonbuffered samples. Higher levels of nitrosamines were formed at 38° than at room temperature. Similar studies with six samples of premixes prepared with or without ascorbate indicated its ineffectiveness in preventing nitrosamine formation. Of 30 samples of various commercial premixes analyzed 17 were positive for nitrosamines. The most commonly found nitrosamines were nitrosodimethylamine, nitrosopyrrolidine, and nitrosopiperidine, all of which are potent carcinogens.

The reported occurrence of traces of N-nitrosamines, many of which are potent carcinogens, in cured meat products has aroused a great deal of concern (Crosby *et al.,* 1972; Sen, 1972; Wasserman *et al.,* 1972). It is generally believed that nitrite, which is added as a preservative, reacts with the amines present in meat, during processing, storage, or cooking, to form these nitroso compounds. The formation of nitrosopyrrolidine (NPy) during frying of bacon (Sen *et al.,* 1973a; Fazio *et al.,* 1973) can be cited as an example. The recent report by Sen *et al.* (1973c) of the occurrence of fairly high levels of nitrosamines in certain types of meat curing mixtures containing spices and nitrite indicates that some of the nitrosamines in cured meat products may originate from these curing mixtures. Nitrosamines in these premixes are formed, apparently under dry condition, due to the interaction of amines in spices and nitrite both of which are major components of these formulations. Subsequently, the use of such premixes has been discontinued both in the United States and Canada.

The rate of formation of nitrosamines from amines and nitrite is dependent on many factors such as the nature of the amines, concentration of the reactants, pH, and temperature of the reaction medium (Mirvish, 1973). In general, the more alkaline the pH, the slower is the rate of the nitrosation reaction. Recent studies (Mirvish *et al.,* 1972; Fiddler *et al.,* 1973) have indicated that, in certain cases, the addition of ascorbic (or erythrobic) acid or its sodium salt can inhibit the nitrosation reaction. Therefore, it was thought that the incorporation of a mild alkali (sodium carbonate) or sodium ascorbate in these premixes

may inhibit the formation of nitrosamines, and thus make them suitable for use. This paper describes the results of such a study carried out in collaboration with two manufacturers of meat curing mixtures in Canada.

MATERIALS AND METHODS

Samples. The samples of the meat curing mixtures were prepared by the two commercial firms according to their own specifications, and shipped to us for nitrosamine analysis. The mixtures mainly consisted of spices or spice extractives, salt, and sodium nitrite. Some of them also contained wheat or corn flour, skim milk powder, tricalcium phosphate (anticaking agent), hydrolyzed plant protein, etc. A brief description of the relevant ingredients in each sample is given in Tables I–III. The buffered samples contained enough sodium carbonate so that a 10% aqueous suspension of the mixture gave a pH of 7.5–8.2. The concentration of sodium nitrite in these premixes ranged between 0.2 and 2%. In addition to sodium nitrite some of them also contained sodium nitrate (0.1–0.96%). Five samples of special premixes analyzed contained only nitrates and no added nitrites.

Nitrosamine Analysis. (a) *Extraction of Nitrosamines.* A 5–25-g aliquot of the sample was moistened by the addition of 5–30 ml of 3 N potassium hydroxide solution and then extracted in a blender for 10 min with 100–300 ml of methylene chloride. During the extraction, about 10–50 g of anhydrous sodium sulfate was added to the mixture to aid the mixing and breaking up of emulsions. The mixture was allowed to settle for a few minutes and the supernatant carefully filtered through glass wool. The filtrate was collected into a 2-l. round-bottomed distillation flask which already contained 100 ml of 3 N potassium hydroxide solution. The extraction was repeated once more, and the entire contents were poured on the funnel containing