(Table I) (Figure 1). There was fairly uniform weight distribution among six other fractions, but none of them attained 11%.

Thin-layer chromatographic separation of the neutral lipids of seven cereal grains has been presented previously (Price and Parsons, 1975). The seven major fractions observed in this study on barley are found in the other six cereals as well. Triglycerides are the principal fraction in every instance. Variation among the seven cereals seems to exist only in numbers of minor fractions labeled unknowns. Sorghum is the widest variant in having two unknowns present in measurable amounts with a higher R_f value than the triglyceride fraction.

The identity of the four unknown fractions has not been established (Table I). They are different from the larger fractions in that they all contain a trace or measurable amounts of arachidic acid (20:0) (Tables II and III). The more slowly ascending unknowns (Figure 1), i.e., unknowns 1, 2, and 3, have a fatty acid distribution that is quite comparable to the other fractions. In these, linoleic (18:2) is the principal fatty acid and palmitic (16:0) is second in percent of total. Unknown 4, a rapidly ascending fraction, contains more higher molecular weight fatty acids, e.g., arachidic, and deviates considerably from the other fractions in fatty acid distribution. In unknown 4, oleic (18:1) is the principal fatty acid and only a relatively small amount of linoleic (18:2) is present.

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Phil B. Price*[1]
John G. Parsons[2]

Agricultural Research
Science and Education Administration
U.S. Department of Agriculture
[1]Plant Science Department
[2]Dairy Science Department
South Dakota State University
Brookings, South Dakota 57007

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Residue Analysis of β-Naphthoxyacetic Acid and β-Naphthol on Field-Sprayed Tomatoes by High-Pressure Liquid Chromatography

Experiments were performed to determine the levels and fate of β-naphthoxyacetic acid (BNOA) and its postulated metabolite (β-naphthol) on tomato flowers, fruit, and leaves when a wettable powder formulation of 42 and 84 ppm active ingredient BNOA in water was sprayed on the tomato plant blossoms in the field, not taking care to avoid leaves and fruit. Residues of BNOA dropped to negligible levels 5–10 days after application and no β-naphthol residues were detected (<0.01 ppm). Even after three spray applications at 10-day intervals, no residue buildup was detected on the plant parts. The tomato leaves retained the BNOA for the longest period of time. However, 5–10 days after application, no significant residues remained on the leaves. Similar results were obtained when the entire plant was intentionally sprayed with BNOA. The harvest fruit in all experiments contained <0.01 ppm of BNOA and β-naphthol.

Various plant growth-regulating hormone-type chemicals such as β-naphthoxyacetic acid, 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid have been investigated as to their effects on tomatoes (Mann and Minges, 1948; Wittwer and Reath, 1952). Emphasis was on early market production rather than on growing the crop for processing.

Tomatoes, which might otherwise be lost due to poor pollination, are set, especially in the early part of the season, by BNOA. Short cloudy days, which lack sunlight and have cool nights, under 15 °C, are conditions that are unfavorable for flower pollination. BNOA stimulates the blossoms to set fruit and holds them on the plants.

The purpose of the present investigation was to determine the levels and fate of β-naphthoxyacetic acid (BNOA) and its postulated metabolite (β-naphthol), if any, on tomato flowers, fruit, and leaves when a wettable powder formulation of 42 and 84 ppm active ingredient BNOA in water was sprayed on the tomato plants in the field. The possibility exists that BNOA could be a useful chemical for assisting in the production of fresh early market tomatoes. Archer and Stokes (1978a) have previously published on the residue analysis of β-naphthoxyacetic acid and β-naphthol by high-pressure liquid chromatography

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(LC) and on the levels of β-naphthoxyacetic acid and β-naphthol on field-sprayed strawberries as analyzed by high-pressure liquid chromatography (Archer and Stokes, 1978b).

MATERIALS AND METHODS

Formulation Applied. Berry-Set, a water-soluble, hormone-type spray powder, Science Products Co., Inc., Chicago, IL, EPA Reg. 2125-39-AA: active ingredient (AI) 2.67% as β-naphthoxyacetic acid (BNOA) and inert ingredients 97.33%. By analysis as described herein, the formulation contained 2.71% BNOA and no detectable postulated metabolite β-naphthol (<0.01%). Two mixtures of this formulation were made: 1.57 g/L to result in a 42-ppm AI solution and 3.14 g/L to result in a 84-ppm AI solution; the mixtures were applied with a Hudson sprayer, 8.5-L capacity. A 0.95-cm i.d. flexible neoprene rubber tubing, Teflon-lined for chemical inertness, was attached between the pressure tank and the spray nozzle.

Tomato Plots. Two rows, each 70 m long and 0.5 m wide, were planted in May 1977 in the University of California at Davis field plots. The tomato plants (Variety UCT-175) were spaced 0.5 m apart within the single rows. Each row was subdivided into plot I (10 plants), plot II (22 plants), plot III (22 plants), plot IV (10 plants), plot V (10 plants), and plot VI (10 plants). The two rows comprised duplicate experiments.

Spray and Sampling of Tomato Plots. The first chemical spray was in July 1977. Plot I was untreated and used as a field control. The tomato plant flower blossoms in plot II were carefully sprayed with short bursts of the 42 ppm BNOA in the water spray, and care was taken to avoid spraying the remainder of the tomato plant. The flower blossoms in plot III were sprayed in the same manner except the BNOA concentration was 84 ppm. Plot IV was sprayed with 84 ppm BNOA in water with a continuous fine spray which covered the entire tomato plant including the flower blossoms and leaves. The spray pattern was repeated on plots I–IV 10 and 20 days after the first spray application of BNOA in the concentrations as described above. Plots V and VI were left untreated until 14 days preharvest which was in August 1977, when ripe tomatoes appeared on the tomato vines. The tomato vines and fruit were then sprayed with a continuous fine water spray containing 42 ppm BNOA (plot V) and 84 ppm BNOA (plot VI).

Samples were taken immediately before and after spraying from plots I–IV on days 0, 10, and 20. Forty blossoms (8 g), 40 leaves (20 g), and 20 tomatoes of each size from each plot were sampled at the appropriate sampling interval. Control (untreated) plots were sampled relative to the treated plots. Samples were also taken on days 5, 15, 25, 30, 35, 42, and 49 days after the initial spraying. There were no tomatoes on the plants on day 0 but blossoms and leaves were sampled. Five days later, blossoms, leaves, and small tomatoes (10–20 g) were sampled. By day 10, the largest fruit had increased in size to 70 g, and two sizes were sampled (small, 10–20 g; medium, 50–75 g). The same approximate two sizes were sampled on day 15. On days 20, 25, and 30, a third large size (100–200 g) was sampled along with the other two approximate sizes (small and medium). After plots V and VI were sprayed on 0 day in August, leaves and ripe tomatoes (150–250 g) were sampled and thereafter on days 3, 5, 7, 14, 21, and 28 days after the single initial spray application. The purpose of this experiment was to study the decline of the BNOA residues on the mature tomatoes even though the chemical is not to be used in this manner. However, during chemical spray applications to the blossoms, the possibility exists that the spray could drift onto large tomatoes present on the vine, resulting in chemical residues on them.

Procedure. *Extraction of the Plant Material.* Fifty grams of tomatoes, 10 g of leaves, or 2 g of blossoms were weighed into a quart stainless steel blender with an explosion-resistant motor cup and extracted as described by Archer and Stokes (1978a). After extraction the tomato extracts were adjusted to 200 mL and the leaves and blossoms to 100 mL with acetone.

A 10-mL aliquot of the sample was added to a 250-mL separatory funnel containing 100 mL of a 10% (w/v) aqueous solution of sodium sulfate. The sample was partitioned three times into 25 mL of dichloromethane which was extracted with three 25-mL aqueous aliquots of 5% sodium bicarbonate (w/v). The combined aqueous fractions were washed with 25 mL of dichloromethane. The combined dichloromethane fractions, containing the β-naphthol, were filtered into a 500-mL, round-bottomed boiling flask. The aqueous combined bicarbonate fractions, containing the β-naphthoxyacetic acid, were acidified with 10 mL of 12 N HCl and extracted with three aliquots of dichloromethane. The combined dichloromethane fractions were filtered into a 500-mL, round-bottomed boiling flask. To each of the boiling flasks was added 10 mL of isooctane, and the volumes were reduced to approximately 5 mL in vacuo at 60–70 °C on a rotary vacuum evaporator.

Acetylation of the β-Naphthol. The β-naphthol fraction was transferred with pentane to a 16 × 150 mm glass-stoppered test tube, and the solvent was removed using a warm water bath and a stream of nitrogen gas. One milliliter of acetylation reagent was added (0.2 mL of 36 N H_2SO_4, 60 mL of acetic anhydride, and 40 mL of glacial acetic acid), and the acetylation reaction was allowed to stand for 1 h at 100 °C in a lightly stoppered glass test tube. The sample was cooled in an ice bath and 2 mL of deionized water was added. The acetylated β-naphthol was partitioned three times into 5 mL of pentane which was stored for cleanup on a Florisil column.

Methylation of the β-Naphthoxyacetic Acid. The β-naphthoxyacetic acid fraction was methylated, cleaned up, and analyzed by LC as described by Archer and Stokes (1978a).

Cleanup of β-Naphthol by Column Chromatography on Florisil. To a 10 × 250 mm glass chromatography column plugged with glass wool was added 3 g of Florisil and 1 g of anhydrous sodium sulfate.

The column was prewet with 50 mL of pentane and the 15-mL sample of β-naphthol in pentane was added to the column, followed by two 5-mL rinses of the test tube onto the column with pentane. The sample was eluted from the column with 250 mL of redistilled pentane. Five milliliters of redistilled isooctane was added to the receiver flask containing the pentane and the solvent volume was reduced to approximately 3 mL using a warm water bath and a rotary evaporator in vacuo at 60–70 °C. The sample was quantitatively transferred from the flask using pentane rinses into a 6.5-mL Mackay–Shevky–Stafford sedimentation tube, the solvent was evaporated to dryness using a warm water bath and a stream of nitrogen gas, and the volumn was adjusted with methanol/water (49:51) for analysis by high-pressure liquid chromatography.

Analysis by Liquid Chromatography. A Tracor liquid chromatograph equipped with a syringe loading sample injector containing a 20-μL sample loop, a variable wavelength ultraviolet light detector set at 223 nm, and containing a 25 cm long, 6.4 mm o.d. × 4.6 mm i.d. column packed with Partisil 10 O.D.S. was used. The mobile phase

Table I. Residues of β-Naphthoxyacetic Acid in Field-Treated Tomato Plants[a]

spray treat-ment no.	expt day no.	residues, ppm											
		blossoms		leaves		tomatoes					
						plot II			plot III		
		plot II	plot III	plot II	plot III	small	medium	large	small	medium	large
1	0	3.26	4.95	0.34	1.18						
	5	0.09	0.17	0.04	0.10	0.10			0.19		
	10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01	
2	10	2.98	4.69	0.76	1.36						
	15	<0.01	<0.01	0.03	0.04	<0.01	<0.01		0.04	0.01	
	20	<0.01	<0.01	0.01	0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01
3	20	3.50	3.48	0.43	1.07		<0.01			<0.01	
	25	<0.01	<0.01	0.06	0.41	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
	30	<0.01	<0.01	0.04	0.10	<0.01	<0.01	<0.01	<0.01	<0.01	
	35	<0.01	<0.01	0.01	0.03			<0.01			<0.01
	42	<0.01	<0.01	0.01	0.03			<0.01			<0.01
	49	<0.01	<0.01	<0.01	0.03			<0.01			<0.01

[a] Only blossoms sprayed with BNOA. Plot II, 42 ppm; plot III, 84 ppm.

Table II. Residues of β-Naphthoxyacetic Acid in Field-Treated Tomato Plants[a]

spray treat-ment no.	expt day no.	residues, ppm				
				tomatoes		
		blossoms	leaves	small	medium	large
1	0	1.77	4.00			
	5	<0.01	1.06	<0.01		
	10	<0.01	0.02	<0.01	<0.01	
2	10	3.93	5.85			
	15	0.23	1.21	0.01	<0.01	
	20	<0.01	0.25	<0.01	<0.01	<0.01
3	20	1.07	4.31		<0.01	
	25	0.04	0.89	<0.01	0.01	<0.01
	30	<0.01	0.22	<0.01	0.02	<0.01
	35	<0.01	0.09			<0.01
	42	<0.01	0.01			<0.01
	49	<0.01	0.01			<0.01

[a] Plot IV. Whole plant sprayed with 84 ppm BNOA.

was 49% methanol in water (v/v) with a flow rate of 72 mL/h with the high-pressure pump set at a stroke of 10 and a frequency of 80. Attenuation was set at 1. Quantitation of the sample peak areas relative to standards was by measurements of peak areas with a polar planimeter. All results were based on fresh weights with a method sensitivity of 0.01 ppm for the compound analyzed. Percent recoveries for BNOA by the analytical procedure were leaves, 98.3; blossoms, 95.6; tomatoes, 94.0; and for β-naphthol, leaves, 71.6; blossoms, 93.0; tomatoes 97.7. No β-naphthol was detected in any sample.

RESULTS AND DISCUSSION

Table I shows the data on blossoms, leaves, and tomatoes on plot II (42 ppm BNOA) and plot III (84 ppm BNOA). The residues of BNOA on all the samples dropped to negligible levels 5 days after the chemical application and no β-naphthol residues were detected (<0.01 ppm). Even after three spray applications of 42 and 84 ppm BNOA at 10-day intervals, no residue buildup was detected on the plant materials. The tomato plant leaves retained the chemical residues for the longest period of time after chemical application. However, after 5–10 days no significant residues remained on the leaves. The tomato fruit had <0.01 ppm residues at harvest 49 days after the initial spray application.

Table II shows the data obtained for residues on the tomato blossoms, leaves, and fruit when the entire plant was intentionally sprayed with 84 ppm BNOA for three treatments at 10-day intervals. Again, no significant residues of BNOA or β-naphthol were detected 5–10 days after the BNOA application. The plant material contained <0.01 ppm residues at harvest, 49 days after the initial spray application, and 29 days after the final spray application.

Table III. Residues of β-Naphthoxyacetic Acid in Field-Treated Tomato Plants and Mature Tomatoes[a]

expt day no.	residues, ppm			
	leaves		mature tomato fruit	
	plot V	plot VI	plot V	plot VI
0	1.94	3.88	0.01	0.04
3	1.15	2.14	<0.01	<0.01
5	0.56	0.66	<0.01	<0.01
7	0.25	0.46	<0.01	<0.01
14	0.10	0.15	<0.01	<0.01
21	0.03	0.10	<0.01	<0.01
28	0.01	0.02	<0.01	<0.01

[a] Whole plants and mature fruit sprayed with BNOA. Plot V, 42 ppm; plot VI, 84 ppm.

Table III shows the data obtained when tomato vines containing mature ripe fruit were sprayed once with 42 ppm BNOA (plot V) and 84 ppm BNOA (plot VI). Tomato vine leaves and mature fruit were sampled for 28 days after the chemical application. Even though this treatment would not intentionally be used on tomato vines, it was of interest to determine the chemical residues on the plant material as well as to investigate the residue decline without plant growth dilution of the residues.

The data shows a gradual decline of BNOA residues on the leaves to insignificant levels within 28 days after the chemical application. With the exception of day 0 of spray application, the tomato fruit residues of BNOA were <0.01

ppm and no β-naphthol residues were detected at any sampling period.

The use of β-naphthoxyacetic acid as a hormone-type fruit set on tomato plants appears safe considering that there were no significant residues (<0.01 ppm) of both BNOA and β-naphthol as shown by the data currently presented.

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Thomas E. Archer*
James D. Stokes

Department of Environmental Toxicology
University of California
Davis, California 95616

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Mechanism for the Mutagenic Activation of the Herbicide Sulfallate

The carcinogenic herbicide *S*-(2-chloroallyl) diethyldithiocarbamate (sulfallate) is metabolized in vitro to 2-chloroacrolein, a potent and direct-acting mutagen in the *Salmonella typhimurium* assay, using strain TA 100. Although not identified, the proximate mutagen may be formed by hydroxylation of sulfallate or one of its dithio- or thiocarbamate metabolites at the methylene group adjacent to the sulfur.

The *S*-chloroallyl thio- and dithiocarbamate herbicides diallate and sulfallate are converted by liver enzymes to potent mutagens (Carere et al., 1978; De Lorenzo et al., 1978; Schuphan et al., 1979; Sikka and Florczyk, 1978). These herbicides are also carcinogens (Innes et al., 1969; National Cancer Institute, 1978) as is often the case with mutagens (McCann et al., 1975). The ultimate mutagen formed from diallate is probably 2-chloroacrolein, resulting from a sequence of sulfoxidation, rearrangement and elimination reactions (Schuphan and Casida, 1979a,b; Schuphan et al., 1979) (Figure 1). We now provide evidence that 2-chloroacrolein is also likely to be the ultimate mutagen formed on sulfallate metabolism.

MATERIALS AND METHODS

Mutagenesis assays were made with *Salmonella typhimurium* strain TA 100 in the presence or absence of the S-9 mix for microsomal activation (Ames et al., 1975).

Sulfallate was obtained from Chem Service (Westchester, PA) and compound 4 (Figure 2) was obtained from K & K Labs (Plainview, NY). The syntheses of compounds **1**–**3** were described by Schuphan and Casida (1979a); the synthesis of 2-chloroacrolein may be found in Schuphan and Casida (1979b). Compound **5** was prepared by refluxing 0.1 mol of 2,3-dichloro-1-propene (Aldrich, Milwaukee, WI) with 10% excess sodium sulfite in 100 mL of H_2O for 1 h and following the work-up procedure given by Schuphan et al. (1977) for the preparation of the sodium salt of (2,3,3-trichloro-2-propene)sulfonic acid. NMR (D_2O, DSS) δ 5.56 (s, $=CH_2$), 3.93 (s, $-CH_2$). [*allyl*-^{14}C]Sulfallate (a gift from Monsanto Co., St. Louis, MO) was used as the substrate for the rat liver microsomal mixed-function oxidase system. Isolation and identification of [^{14}C]-2-chloroacrolein was as previously described (Schuphan and Casida, 1979b); this trapping system was probably of relatively low efficiency so the findings are more qualitative than quantitative in nature.

RESULTS

Figure 2 compares the mutagenic potencies of sulfallate and 2-chloroacrolein with those of three sulfallate derivatives (**1**–**3**) formed easily on oxidation (Schuphan and Casida, 1979a) and two other potential metabolites (**4** and **5**). Except for 2-chloroacrolein, each compound is inactive or requires the S-9 mix for detection or enhanced activity. The mutagenic potency with S-9 mix decreases in the order 2-chloroacrolein ≫ sulfallate ≫ **1** ≫ **2** = **3** > **4** > **5**.

2-Chloroacrolein was established as a microsomal oxidase metabolite of sulfallate by trapping this aldehyde as its 2,4-dinitrophenylhydrazone derivative. The amount recovered was 0.4 and 0.8% (two experiments) with the oxidase cofactor, NADPH, and 0.01% for the control without this cofactor.

DISCUSSION

Two types of evidence strongly indicate that 2-chloroacrolein is the ultimate mutagen formed during sulfallate metabolism. First, it is the only likely metabolite of the chloroallyl moiety with sufficiently high mutagenic activity (Rosen et al., 1980) to be a candidate for the ultimate mutagen. Second, 2-chloroacrolein is liberated from sulfallate in the microsomal oxidase system. It is therefore of interest to consider the nature of the proximate mutagen.

2-Chloroacrolein is easily formed from diallate by sulfoxidation followed by [2,3] sigmatropic rearrangement and 1,2-elimination reactions. However, this sequence is not applicable to sulfallate because it lacks the 3-chloro substituent necessary for the final elimination reaction to form an aldehyde (Schuphan and Casida, 1979a). Sulfallate oxidation and rearrangement forming **1**, **2**, and **3** yields no direct-acting mutagen. Each of these compounds requires metabolic activation for mutagenic activity.

2-Chloroallyl alcohol, a possible intermediate to 2-chloroacrolein, may be formed by several pathways (Figure 2), including hydrolysis of **3** and metabolism of 2-chloroallyl mercaptan. It is difficult to accept metabolic routes via 2-chloroallyl alcohol as major contributors to sulfallate activation since the herbicide is 145 times more potent as a mutagen than this alcohol.

Metabolic activation of sulfallate must occur by a pathway(s) of relatively high efficiency in forming 2-chloroacrolein. This would be the case for α-hydroxylation of sulfallate followed by immediate decomposition of the α-hydroxy compound to 2-chloroacrolein (Figure 2). An α-hydroxy intermediate may also be involved with a variety of other compounds including **1**, **3**, and 2-chloroallyl mercaptan. Previous studies on thiocarbamates have established the importance of α-hydroxylation in aldehyde liberation from the thiol moiety. About 7–17% of the

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