Exposure of Pesticide Applicators to Nitrofen: Influence of Formulation, Handling Systems, and Protective Garments

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Ten pesticide applicators in Michigan, Wisconsin, and Minnesota were monitored for dermal and inhalation exposure to nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) during typical mixing and spraying operations on onion, carrot, cabbage, and celery crops. The variables studied were formulation [emulsifiable concentrate (EC) vs. wettable powder (WP)], handling (open vs. liquid pumping), body location (palm, leg, arm, chest, head), and protective garments (best protection vs. minimal protection scenarious). Handling the WP formulation provided the highest potential dermal and inhalation exposure, much of which occurred during the mixing operation. Nitrofen deposits on the hands, a major site of dermal exposure, could be reduced by an average factor of approximately 220 by using rubber gloves. Potential daily exposure was reduced more than half by using the EC rather than the WP formulation, even if no protective garments were worn. Total exposure was reduced approximately 78% by pumping vs. pouring the EC formulation and more than 90% by handling pumped EC compared to WP. Daily exposure can be reduced to less than 300 μg when wearing a protective coverall plus an air filtration system and when handling the EC formulation.

The herbicide nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) has provided unique selective weed control properties in a number of intensively managed vegetable and ornamental crops. For many of these crops it has been the only registered herbicide with activity on emerged weeds. Nitrofen has high economic value to the producer of these specialty crops. For example, in onion production, its use at 4 times during the season at 1.0 lb/acre can eliminate an average of 76 h of hand labor per acre and save up to an additional 36 lb/acre in alternative herbicide usage (Boldt et al., 1981).

Prior to 1981, nitrofen was marketed as a 50% wettable powder (WP) or 25% emulsifiable concentrate (EC) formulation. The WP was packaged in 5-lb paper bags and the EC was sold in 5- or 35-gal metal cans. The Rohm and Haas Co. voluntarily discontinued marketing nitrofen prior to the 1981 season after several studies had disclosed potential toxicology problems with the compound.

At least three studies have indicated that nitrofen may cause tetratogenic effects if female rats or mice are exposed during gestation (Ambrose et al., 1971; Kimbrough et al., 1974; Gray et al., 1982). Other animal studies have shown nitrofen to be a possible mutagen and carcinogen (Paik and Lee, 1977; Milman et al., 1978; National Cancer Institute, 1978). Another potential concern is the relatively high dermal absorption rate (Burke, 1981). No serious human illnesses, birth defects, or deaths have been attributed to exposure to this compound.

Sensitive EC/GLC methods have been developed for detection of nitrofen and its metabolites in crops and soils (Adler and Wargo, 1975; Wargo et al., 1975; Honeycutt and Alder, 1975). Nitrofen is degraded rapidly in both plants and soil and is also subject to rapid adsorption on soil colloids (Fadayomi and Warren, 1977).

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A previous California exposure study (Maddy et al., 1980) indicated that dermal greatly exceeded inhalation exposure and that handling the WP formulation provided more exposure than handling the emulsifiable concentrate. They estimated that an applicator in a 5-h day (1-h mixing–loading, 4-h spraying) might receive exposure to 7.23 and 3.00 mg of each formulation, respectively.

The objectives of this study were to determine the potential dermal and inhalation exposure of mixer–applicators who spray vegetable crops in the Midwestern United States. In addition, the effectiveness of protective garments and air filtration systems was ascertained for handling nitrofen by three different methods.

EXPERIMENTAL SECTION

Cooperators. The 10 cooperators were selected to represent a cross section from mid-western vegetable industry applicators who had previously applied TOK (formulated nitrofen) to vegetables grown on muck soils. The applicators were all males ranging from 26 to 48 years of age who routinely apply pesticides with ground equipment (Table I). They were informed of the objectives of this experiment and advised in advance that protective clothing would have to be worn. Otherwise, they agreed to handle the chemicals as they normally would in their own operations. The crops sprayed were cabbage, carrots, celery, and onions which are the major crops upon which nitrofen was utilized in the Midwest. All the tests were conducted in July and Aug of 1981. The application equipment included tractor or trailer-mounted sprayers, some of which were homefabricated while others were commercially built (Table I). The applications were made in spray volumes of 18–50 gal of water/acre. Temperature ranged from 24 to 34 °C during application, while winds were from 0 to 12.8 km/h.

Handling Systems and Formulations. Three systems of handling were compared. The 5-lb bags of TOK–50% wettable powder (WP) were opened by the mix applicator, poured into a plastic bucket, and stirred to make a slurry with water. The slurry was then poured into the spray tank.

The emulsifiable concentrate (TOK E-25) was handled by two different methods. The proper quantity for a tankful was either poured from a 5-gal metal can into a smaller plastic measuring container and added to the tank

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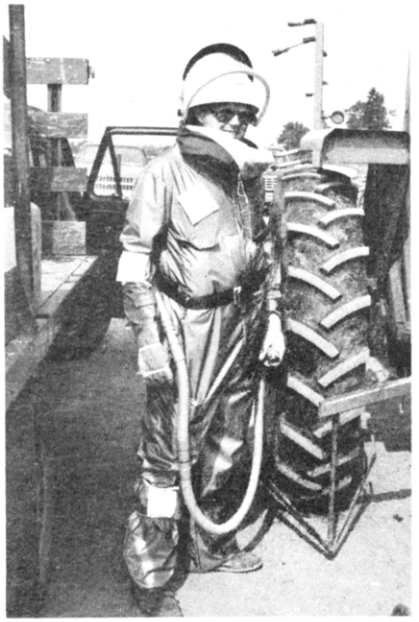

Figure 1. Applicator with Gore-Tex coverall especially designed and constructed for this study. Note location of gauze sampling pads on leg, arm, chest, and inside palm.

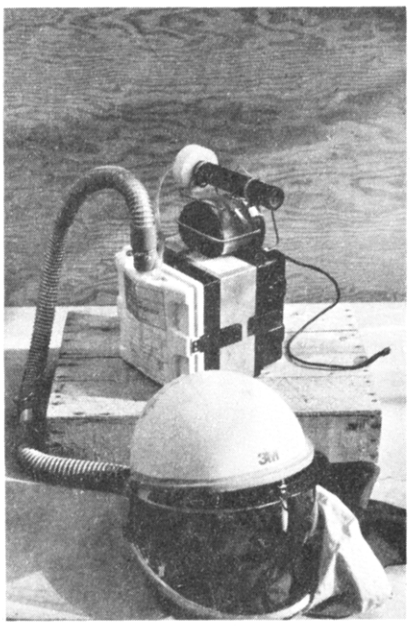

Figure 2. The 3M-Whitecap and Model W-2801 air purification system used by applicators in this study. Attached to this unit is an Alltech Model 1540 air pump to sample ambient and purified air.

or pumped directly from a 35-gal barrel with a Tuthill series 5200 pump and 800-A meter. At each location the applicator chose a rate suitable for the weed problem and crop involved. The rates varied from 1.0 to 2.0 lb (active ingredient)/acre and were kept constant across formulations within a location. The sequence of handling methods for each site is shown in Table I.

The time required to accomplish each operation, i.e., mixing and loading, travel, and spraying, was recorded for handling method and applicator. In all locations except one, the samples represent total exposure during all three operations. With one cooperator (no. 8), exposure during mixing and loading was sampled separately from that of traveling and spraying.

Protective Garments. Each applicator was provided a protective coverall fabricated especially for this study (Figure 1). The garments were constructed with a Gore-Tex Teflon barrier sandwiched between two layers of "Rip Stop" nylon. The fabric had proven to be virtually impenetrable to a variety of pesticides in previous tests (Orlando et al., 1981). Three pockets were sewed into the interior of the garments which served as repositories for coolant packs (Temp Aid) to help keep the applicator comfortable in hot weather.

The applicators were also furnished rubber gloves (Edmont 26-680) and were provided the option of wearing 15-in. high rubber boots or their own boots.

To reduce inhalation exposure, the applicators were provided a 3M White Cap helmet attached to a Model W-2801 air purification system (Figure 2). This unit contains a series of particulate filters and an adsorption cartridge designed especially for pesticides. Each unit was equipped with approximately 30 ft of hose to allow freedom of movement while mixing and loading. The air purification unit was attached to the tractor and powered by a 12-V battery during the spraying operation.

One applicator (3) utilized a closed-cab tractor that contained its own air purification (particulate and charcoal) system (Figure 3). In this instance, the air sampling unit was placed inside his cab to monitor its effectiveness.

Sampling Techniques. The sampling strategy was to determine exposure in our best-protection scenario (inside the protective clothing and helmet) and a no-protection

Figure 3. Closed-cab tractor utilized by applicator no. 3. In this case air samples were collected inside the cab.

scenario (outside the protective clothing). Dermal sampling was accomplished by preparing foil-backed gauze pads 10.2 by 10.2 cm or 5.1 by 5.1 cm in size. The 10.2 by 10.2 cm pads were attached to the chest, lower leg, forearm, and head inside or outside the protective garment or helmet. A 5.1 by 5.1 cm gauze pad was also attached inside and outside of the rubber gloves with elastic fabric bands around the hand and wrist. The pads were always positioned, handled, and collected by the experimentors, although the applicator was allowed to remove his own protective garments. Care was taken to avoid accidental contamination of pads by keeping them sealed in plastic before and after use. After the applicator completed mixing and/or spraying, the pads were immediately removed, placed in individual plastic bags, and put directly onto ice for transport to the laboratory freezer. All samples were frozen (−20 °C) within 3 h after collection.

Inhalation exposure was monitored by collecting air samples throughout the mixing, loading, traveling, and spraying operations. The air was passed through glass tubes (7 by 70 mm) packed with 150 mg of acetone-washed XAD-4 (Rohm and Haas Co.) resin. These columns had previously been reported to trap nitrofen at high efficiencies (Burke, 1981). Air samples were collected from

Table I. Cooperators and Other Descriptive Information Regarding the Test Sites

applicator	location, state	position	age	crops sprayed	sprayer (boom location)[a]	tank size, gal	spray volume, gal/acre	lb/acre (a.i.)	av air temp, °C	av wind velocity, km/h, direction	order of handling	time of exposure, min[b]
1	Stockbridge, MI	owner-applicator	46	onion	FMC, trailer mounted (rear)	250	50	1.5	28.3	0	EC-C	20.7
											EC-O	14.1
											WP	14.0
2	Bath, MI	manager-applicator	33	carrot, onion, cabbage	homemade, tractor mounted (rear)	85	40	2.0	23.3	8.0, SW	EC-O	16.3
											EC-C	14.3
											WP	20.5
3[c]	Hudsonville, MI	applicator	40	celery	homemade, trailer mounted (front)	120	25	1.5	28.3	4.8, NW	EC-C	18.4
											EC-O	15.2
											WP	15.0
4	Stockbridge, MI	applicator	27	onion	FMC, tractor mounted (front)	200	50	1.25	25.0	4.8, W	EC-C	18.6
											EC-O	19.0
											WP	16.4
5	Sheridan, MI	applicator	27	onion	homemade, trailer mounted (rear)	500	60	2.0	29.9	7.2, S	EC-C	18.4
											EC-O	16.4
6	Hudsonville, MI	owner-applicator	32	celery	Myers, tractor mounted (front)	120	40	1.0	29.9	3.2, NW	EC-C	27.5
											EC-O	19.3
											WP	19.9
7	St. Paul, MN	applicator	26	no crop	homemade tractor mounted (front)	30	18	2.0	31.6	6.4, SW	EC-O	11.8
											EC-C	11.1
											WP	14.3
8	Hollandale, MI	applicator	30	onion	Demco, trailer mounted (rear)	300	20	1.0	29.4	0	EC-O	18.9
											EC-C	21.9
											WP	22.5
9	Palmyra, WI	applicator	48	carrot	homemade, tractor mounted (front)	240	40	2.0	26.6	12.8, NW	WP	37.0
											EC-O	38.0
											EC-C	41.0
10	Berlin, WI	applicator	40	celery	homemade, tractor mounted (rear)	50	50	2.0	24.9	4.0, SW	EC-O	33.0
											WP	18.0
											EC-C	20.0

[a] Front or rear of tractor operator. [b] Includes mixing, loading, traveling, and spraying. [c] Used closed-cab tractor with air purification system.

Table II. Averages and Ranges of Dermal Exposure As Influenced by Protective Garments and Handling Methods

handling system[a]	location to garment[a]	ng/cm²				
		palm	leg	arm	chest	head
EC	inside	43.7 (15.0-73.5)	14.2 (2.6-25.5)	12.7 (3.0-30.6)	14.3 (1.7-69.6)	13.0 (1.6-28.6)
pumped	outside	78.0 (3.7-2247)	86.3 (6.0-368)	66.0 (7.6-247)	42.2 (6.9-151)	59.2 (4.4-200)
EC open	inside	39.6 (1.6-94.0)	12.1 (3.8-19.1)	14.9 (5.8-40.5)	16.4 (2.2-38.8)	13.7 (1.4-46.0)
	outside	17 000 (69-142 200)	50.2 (4.7-166)	64.3 (1.7-180)	147.0 (4.4-1185)	838.0 (6.3-6708)
WP	inside	52.4 (9.8-116)	22.7 (3.8-50)	29.6 (2.8-191)	20.3 (3.3-111)	15.8 (3.9-47.8)
	outside	14 960 (64-63 740)	2202 (6.3-18 870)	233.9 (16.2-1230)	99.8 (10.6-494)	135.9 (7.6-915)

[a] F values from analyses of variance indicated that (1) exposure to all body parts was significantly reduced at $P = 0.01$ by protective garment and (2) WP formulation provides more exposure than either EC system at $P = 0.05$.

outside and inside the filtering system. The air was sampled at a constant rate by attaching a small portable air pump (Alltech No. 1540; Figure 2) to the air purification unit. The flow rates were monitored prior to each run and approximately 4000 ± 500 cm³/min. The tubes were capped and immediately placed on ice and stored in the freezer prior to analysis. Fortified standards, field blanks, and spikes were also run to determine efficacy of extraction and check on possible contamination.

Residue Analyses. Prior to extraction, the larger gauze pads were trimmed to 5 by 5 cm and the smaller ones (palm samples) to 2.5 by 2.5 cm. The analyses were performed generally as previously described (Burke, 1981). The pads were placed into soxhlet extraction tubes and extracted 3 h in 300 mL of dichloromethane with a minimum of 10 extraction cycles occurring during the 3-h period. The extracts were evaporated to dryness under reduced pressure in a 45 °C water bath. The nitrofen residues were transferred to a Florisil column with 10 plus 30 mL of petroleum either. the column consisted of 10 mL of 5% water-deactivated Florisil (100–200 mesh) topped with 2 mL of sodium sulfate in a disposable 10-mL pipet. Nitrofen was eluted from the column with 80 mL of 5% (v/v) diethyl ether in petroleum ether, after which the effluent was evaporated to dryness. The nitrofen was redissolved in 25 mL of toluene and diluted as required for ECD/GC analysis. GC was accomplished with a Shimadzu Model GC-4 CM equipped with an ECD. The 2.0 m by 2.6 mm packed glass columns contained 10% OV-11 on 100–120-mesh Suplecoport. Column and injector–detector temperatures were 235 and 270 °C, respectively. Injection volumes were 6 μL. Under these conditions, the retention time for nitrofen was 7.5 min. The chart speed was maintained at 1.27 cm/min and quantitation was achieved by peak height measurement. Standard curves were obtained from nitrofen standards containing 1.25, 2.50, 5.00, and 9.90 ng/mL. Analysis of gauze pads spiked with [^{14}C]nitrofen showed that this method recovered an average of 97.9% of the quantity applied.

The XAD-4 tube contents were placed in 5 mL of acetone in 20 by 150 mm culture tubes with screw caps. The culture tubes were agitated for 1-h on a wrist action shaker, after which a 1.0-mL aliquot was transferred to a vial and dried under nitrogen. The residue was redisolved in 2 mL of toluene, and 6-μL aliquots removed for analysis. Recoveries from XAD-4 tubes spiked with [^{14}C]nitrofen were 94% efficient.

RESULTS AND DISCUSSION

Dermal Exposure. The major potential exposure area to nitrofen was the hands, which without rubber gloves could have recieved relatively high dosages (17.1 and 14.9 μg/cm², respectively) with the 5-gal EC and 50% WP handling systems (Table II). With the liquid pumping system, the potential exposure to hands was reduced approximately 20-fold. The rubber gloves utilized in this study did not completely eliminate potential exposure but greatly reduced it by factors up to 300:1. Exposures to the hand (per cm²) were thereby reduced to levels only 2–4 times that above other parts of the body, which were all extremely low inside the protective garments. Furthermore, other workers have indicated that butyl rubber may provide even better protection than the natural rubber from which these gloves were fabricated (Burke, 1981).

The variablity in data among applicators was large. This might be expected because of inherent differences in handling techniques and differences caused by weather, i.e., wind, position in relation to spray boom, etc.

As might be expected, the amounts of nitrofen contacting the protective garments were greatest with the WP formulation and least with the liquid pumping system (Table II). Liquid pumping reduced exposure to the outside of the garment by a factor of approximately 5 when compared to WP and EC open handling systems. The protective coverall and helmet were extremely effective in preventing exposure to all parts of the body that were monitored. They provided up to 100-fold protection when comparisons were made to deposits that occurred outside the garments. When the EC formulation was utilized, consistently lower values were obtained inside the garment for the leg, arm, chest, and head areas. The values were only slightly higher when the WP formulation was used.

One of the questions that always arises in these studies is what component of the operation contributes most to exposure. For one applicator (no. 8), data were obtained to compare the mixing and loading component with the spraying component across all handling systems and exposure sites (Table III). There was consistantly less exposure inside the garments when handling EC vs. WP formulations. Potential exposure when handling the EC or WP formulations in open systems occurred primarily during the mixing and loading operations. The potential for exposure during mixing and loading the WP was extremely high but could be reduced by factors up to 1000 with proper protective garments. These data clearly indicate that a liquid pumping system can greatly reduce dermal exposure to nitrofen, and presumably other pesticides as well, during mixing and loading operations. Another important conclusion obtained from this data is that protective garments can provide excellent protection from dermal exposure even when the potential for exposure is extremely severe as in the case of the WP in this study. The minute quantities of nitrofen that were detected inside the protective garments may have entered through the open sleeves or legs. It is possible that insertion of elastic in these two areas which would provide a tighter fit to the wrist and ankle could further reduce exposure.

In some instances, higher residues were found on the inside pads. An additional source of exposure on the inside pads may accrue when the applicator desuits. If he handles the outside of the fabric or brushes it against the body,

Table III. Comparison of Average Exposure in a Mixing and Loading vs. a Spraying Operation[a]

system[b]	location to garment	operation	ng/cm² palm	leg	arm	chest	head
EC pumped	inside	mixing + loading	31.4	5.6	4.7	5.2	7.6
		spraying	34.0	19.9	17.2	64.4	4.5
	outside	mixing + loading	37.3	0.6	8.2	87.8	196.1
		spraying	347.3	4.5	7.6	6.9	4.6
EC open	inside	mixing + loading	1.6	4.0	5.8	2.2	2.8
		spraying	3.6	11.4	34.7	6.0	1.4
	outside	mixing + loading	8466	4.7	77.6	4.4	6701
		spraying	15.0	–[c]	1.7	6.4	7.0
WP	inside	mixing + loading	60.5	18.4	137.9	91.9	40.8
		spraying	34.0	19.9	53.1	19.2	7.0
	outside	mixing + loading	33100	18180	980.6	449.4	847.8
		spraying	9806	686.4	249.2	44.9	67.4

[a] Applicator no. 8. [b] Analysis of variance indicates (1) more exposure from mixing and loading operations involving WP and EC handled open vs. the EC pumped, (2) more exposure from spraying WP than EC open or pumped, and (3) less exposure inside garments when handling EC vs. WP formulation. All significant at $P = 0.01$. [c] Sample lost.

Table IV. Averages and Ranges of Inhalation Exposure (ng/L) to Nitrofen As Influenced by Air Filtration and Handling Methods

handling system	location to air filtration	av area range,[a] ng/L	frequency of detection
EC pumped	inside	1.78 (ND-33.6)	0.3
	outside	112.6 (ND-595)	0.6
EC open	inside	3.15 (ND-15.0)	0.3
	outside	17.73 (ND-40.2)	0.6
WP	inside	33.23 (ND-287)	0.5
	outside	3307 (ND-16 457)	0.5

[a] Analysis of variance indicates (1) values outside the filter differ significantly from those inside the filter at $P = 0.01$ and (2) averages for WP differ significantly from EC at $P = 0.05$.

Table V. Estimated Daily Exposure to Nitrofen As Influenced by Handling Method and Protective Garments

handling method	exposure type	estimated daily[a] exposure to nitrofen, μg inside[d] garment or helmet	outside[d] garment or helmet
EC pumped	dermal[b]	214.7	3 215
	inhalation[c]	11.1	701
	total	225.8	3 916
EC open	dermal	227.8	17 610
	inhalation	19.7	110.8
	total	247.5	17 720
WP	dermal	327.3	19 370
	inhalation	207.7	20 670
	total	535.0	40 040

[a] Based on a 5-h work day (1-h mixing-loading, 4-h spraying). [b] Calculated on the basis of the following surface areas (cm²): trunk = 7030; arms = 2498; legs and feet = 2030; head and neck = 1100; hands = 900. [c] Inhalation based on a breathing rate of 1.25 m³/h. [d] Analysis of variance indicates (1) daily exposures from WP are significantly higher than those from EC at $P = 0.01$ and (2) daily exposures outside protective garments are significantly higher than those inside at $P = 0.01$.

it could produce additional residues. We allowed the applicators to remove their own protective garments as they normally would in the course of their work. Other studies that have neglected this component of exposure may be somewhat unrealistic.

Inhalation Exposure. Although inhalation exposures were extremely variable, some trends were evident (Table IV). These are perhaps best shown by observing the frequencies for which residues were detected and the mean levels detected under each handling system. Outside of the air filters, residues were detected in 17 of 36 samples whereas inside they were detected in only 10 samples. The highest mean residue level occurred in samples where the WP formulation was handled. The filters reduced residues by factors ranging from 5.6 to 100. The only extremely high values obtained in the entire study were those obtained during mixing and loading operations. Since detectable levels were found in only half the samples, even outside the filters, the potential for inhalation exposure appears lower than that for dermal exposure. Significant inhalation exposure apparently occurs only when the wettable power is handled and then only under certain handling conditions. For example, half the applicators tested handled the wettable powder without detectable inhalation exposure. A closed tractor cab equipped with particulate and adsorptive filters appears to be a practical and effective alternative to the helmet system during the spraying operation.

CONCLUSIONS

The average exposures (ng/cm²) indicated in Tables II, III, and IV were summarized and converted to total exposures that could be expected for an average 175-lb male (Table V). To accomplish this, estimated surface areas for various body parts were adopted from Hayes (1975). A 5-h exposure day was assumed (1-h mixing–loading, 4-h spraying). Without protective garments, potential daily exposure when handling either the EC from 5-gal cans or the 50% WP was approximately 17.7 and 40 mg, respectively. Obviously, the clothing worn under these protective garments could provide additional protection. In contrast, the liquid pumping system provided approximately 90% reduction in exposure even without protective garments. With protective coveralls, gloves, and helmet equipped with the air purification system, exposure was greatly reduced with all handling systems. Liquid pumping with protective garments reduced exposure to an average of 226 μg/day. The trunk and lower limbs contributed about equal portions of the total exposure (35–40%) primarily because of their larger surface areas. Some of this may have occurred when the applicators desuited. With no protective garments, the hands would contribute by far the greatest exposure.

These data indicate that excessive exposure might be expected by some applicators who handle either WP or EC without effective protective garments. Total exposure can be greatly reduced by handling an EC formulation that is pumped and by using protective garments similar to those utilized in this study. Exposure might be even further

reduced by slight modifications in these protective garments, such as elastic in sleeves and exclusive use of butyl rubber gloves and boots.

The Midwestern applicators who were sampled indicated that they would never handle this product more than 10 days/season. Assuming this handling frequency, and use of the pumped-EC formulation in our best protection scenario, average exposure for these applicators should not exceed 2500 μg/season.

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A Simple Method for Purification and Determination of Miserotoxin

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A column chromatography method was developed to simplify miserotoxin (3-nitro-1-propyl β-D-glucoside) purification from timber milkvetch (*Astragalus miser* var. *serotinus*). The column eluate was then directly treated with diazotized *p*-nitroaniline to determine the concentration of the toxic glycoside in the forage. The column method agreed favorably ($r = 0.97$) with the GC procedure for miserotoxin determination, but erratic values were obtained when the glycoside was estimated with the Griess-Ilosvay reagent after KOH displacement of the nitro group. Aqueous extracts of fresh plant material contained factors that enhanced the yield of nitrite during KOH treatment. The interference was reduced in acid extracts of oven-dried plant samples, but the latter treatment also reduced the yield of miserotoxin.

Aliphatic nitro compounds occur in over 500 *Astragalus* species of the family Leguminosae (Williams, 1981a). The compounds also occur in other legume genera (*Coronilla*, *Indigofera*, and *Lotus*) and, less abundantly, in other families (Williams, 1981b). Derivatives of 3-nitropropanol (NPOH) or 3-nitropropionic acid (NPA) are usually detected. Glucose esters of NPA and the β-D-glucoside of NPOH (miserotoxin) have been isolated and identified, but NPA and NPOH do not occur together (Stermitz and Yost, 1978). Numerous studies have demonstrated the toxicity of NPA or NPOH to ruminant and nonruminant animals (James et al., 1980; Majak et al., 1981; Shenk et al., 1976). In British Columbia, timber milkvetch (*Astragalus miser* var. *serotinus*) is widely distributed on Interior grassland and forest range and miserotoxin can accumulate to levels exceeding 8% of the dry herbage weight (Majak et al., 1977).

Derivatives of NPA and NPOH can be determined spectrophotometrically by direct coupling of diazonium salts to the aci tautomers (Majak and Bose, 1974) or indirectly by measuring the nitrite ion after alkaline displacement of the nitro group. The Griess-Ilosvay reagent determines nitrite by coupling 1-naphthylamine to the diazonium salt formed with sulfanilic acid (Bose, 1931). When this reagent is used, the sensitivity of the procedure depends essentially on the yield of nitrite ion. An improved procedure for liberating nitrite at pH 9.5 (Matsumoto et al., 1961) is widely used for NPA because the yield of nitrite exceeds 90%. The yield is much lower if NPA is treated with 20% KOH (Cooke, 1955). In spite of this, a slightly modified version of Cooke's method (Williams and Norris, 1969) appears to be effective for screening purposes (Williams and Barneby, 1977), and it was also used for estimating miserotoxin levels (Parker and Williams, 1974).

As reported earlier (Majak and Bose, 1974), the Griess-Ilosvay reagent was not suitable for miserotoxin analysis due to interfering substances and low yields of nitrite in crude extracts of timber milkvetch. This led to the development of the direct coupling system for miserotoxin determination, but a partial TLC purification was required for plant extracts prior to spectrophotometric

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