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Worker Exposure to Diazinon During Flea Control Operations in Response to a Plague Epizootic

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Plague is an acute and potentially fatal bacterial infection caused by *Yersinia pestis* that affects humans and animals. In California, plague is maintained in an enzootic cycle among wild rodents and their associated fleas. Fleas associated with ground squirrels and chipmunks are considered to be the primary vectors of plague from rodents to humans (Barnes 1982).

Plague control operations to protect human health in California are conducted by public health agencies and may involve the use of insecticides to reduce rodent flea populations in localized areas (Smith 2002). One commonly used treatment method involves the placement of insecticidal dust in and around the entrance to rodent burrows. As rodents enter or exit treated burrows, some insecticide adheres to the body, indirectly using the animal as a vehicle for precise insecticide placement inside the burrow system where the majority of adult and immature fleas are found. Used in this way, a relatively small quantity of insecticide may be effective in reducing flea numbers over a large geographic area.

Over the last decade, public health agencies have experienced a dramatic reduction in the availability of chemical products for the control of public health pests (Rose 2001). This reduction stems from registration and use restrictions placed upon these products by the U.S. Environmental Protection Agency (USEPA), in part as a result of perceived human risk associated with these products. Measurements of human exposure to insecticides used for control of public health pests have not been conducted, leaving the USEPA to calculate human exposure risk using very conservative assumptions and/or from limited exposure data from the use of these insecticides in agriculture.

This study provides an initial assessment of worker exposure data resulting from the use of an organophosphate insecticidal dust (diazinon) by trained and certified public health pesticide applicators during plague control operations.

MATERIALS AND METHODS

During August of 2002, flea control operations to reduce the risk of plague transmission to humans were conducted at a temporarily closed public

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campground in Inyo County, CA where plague infected ground squirrels had been captured the previous month. Five certified public health pesticide applicators (workers) from the California Department of Health Services and the Inyo County Department of Environmental Health applied 2% diazinon dust (Gold Crest Diazinon 2D Insecticidal Dust, Roussel Bio Corporation, Englewood, NJ) to rodent burrows under provisions of the California Special Local Needs Permit #CA-800157 to the Department of Health Services. All five workers had a minimum of three years experience conducting flea control operations.

Workers wore protective clothing consisting of Tyvek® coveralls, N100 particulate respirators (No. 8233, 3M Corporation, St. Paul, MN), surgical gloves, and goggles or eye glasses. Workers applied diazinon dust via hand-held pump dusters (Dust-R, model 1152-A, B&G Equipment Co., Plumsteadville, PA) to the entrance of ground squirrel and chipmunk burrows within the campground. Pump dusters were filled with diazinon dust by only two of the five workers, whereas all five workers applied diazinon dust to rodent burrows. Loading the pump dusters required that diazinon dust be spooned from an 11.36 kg container into 1.35 kg pump dusters. Over the course of the control operation, each pump duster was filled with diazinon dust four times (one initial loading and three refills) by the two loaders.

The entire flea control operation required 165 min, of which 103 min comprised actual product application time and 62 min comprised the time for loading of pump dusters and movement (by walking) to treatment areas. In total, 9.14 kg of diazinon dust was applied by 5 workers to 438 rodent burrows (Table 1).

To estimate diazinon exposure, metabolites of the parent compound excreted in the urine [diethylphosphate (DEP) and diethylthiophosphate (DETP)] were used as biomarkers to indicate how much of the parent compound was absorbed and metabolized. The biomarker diethyldithiophosphate (DEDTP), which is not a metabolite of diazinon, was also screened to determine whether workers were concurrently exposed to other common organophosphates for which DEDTP is a metabolite.

Pre-exposure (control) urine samples for each worker (No. 0010-0050) consisted of 5-10 ml of urine from the first void on the morning prior to pesticide application (day 0). Thereafter, urine samples consisting of 5-10 ml of urine from the first morning void each day were collected by each worker for five consecutive days (days 1-5). Urine was collected into a 10 ml tube and retained on wet ice until the following week when samples were placed at -80°C and held until processing.

Urine samples were analyzed for diethylphosphates (DEP, DETP, DEDTP) and creatinine using procedures developed in cooperation with the Pacific Toxicology Laboratory, Chatsworth, CA. Five ml aliquots of each urine sample were freeze-dried, suspended in acetone containing the derivatizing agent 3-benzyl-1-p-

Table 1. Amount of 2% diazinon dust applied and the number of rodent burrows treated by each worker.

Worker	Amount Dust Applied (g)	Rodent Burrows Treated[1]
0010*	1895.6	93
0020*	1895.6	42
0030	1489.4	54
0040	2166.4	147
0050	1692.5	102
Total	***9139.5***	***438***

[1] Diazinon dust was applied to each rodent burrow with two pumps of a commercially available pump duster. It was noted with 5 test applications that the amount of product discharged by two pumps varied from 4.9 to 16.6 g (mean = 10.74 ± 5.24 g), affected by both the force applied to the plunger handle and the angle at which the duster was held.
* Workers responsible for loading pump dusters for all crew members.

tolyltriazine, held for 2 hr at 70°C, and then held overnight at room temperature. Excess derivatizing agent (3-benzyl-1-p-tolyltriazine) was added to each sample to ensure complete conversion of the diethylphosphates. Benzyl derivatives were extracted concurrently with an internal standard (fenthion) from an aqueous salt solution using cyclohexane. All derivatives were analyzed using HP 5890 GC with flame photometric detector (FPD) and a 30 M DB-210 column. The temperature program was: 250°C for the injection port, 160°C for the column which was held for 6 min with temperature increasing thereafter by 5°C/min to 190°C, followed by a more rapid temperature increase of 30°C/min to a final temperature of 230°C which was held for 7 min. The detector temperature was 250°C. The helium flow rate was 10 ml/min. Recoveries from spiked urine specimens were 108% for DEP, 90% for DETP, and 108% for DEDTP. The limit of quantification (LOQ) using this technique was 5 ppb for DEP and DETP, and 10 ppb for DEDTP. Those measurements below the LOQ were treated as half of the LOQ.

The absorbed daily dose and dosage (dose adjusted for body weight) of diazinon for each worker were based on the quantification of diethylphosphates extracted from each of 5 urine samples provided by the workers. Extracted diethylphosphates in each sample were transformed using the daily creatinine conversion factor of 1.7 g creatinine/day (Snyder 1994) as below:

Daily dose of diazinon (μg/day) =

$$\left(\frac{\text{DEP } (\mu\text{g/L})}{\text{FW DEP (154)}} + \frac{\text{DETP } (\mu\text{g/L})}{\text{FW DETP (170)}}\right) \times \text{FW diazinon (304)}$$

$$\div \text{ urinary creatinine concentration (g/L)} \times 1.7 \text{ g creatinine/day}$$

Daily dosage of diazinon (μg/kg-day) = daily dose (μg/day) ÷ body weight (kg)

Cumulative or aggregate diazinon exposure (absorption) was calculated as the sum of the five consecutive post-exposure daily dose values. Statistical comparisons were made using a one factor repeated measures ANOVA procedure with means separated by Duncan's Multiple Range Test (Cody and Smith 1997).

RESULTS AND DISCUSSION

Quantification of breakdown metabolites present in urine samples indicated that all workers had very low levels of diazinon exposure, and in many of the samples the presence of diazinon breakdown products was undetectable (Table 2). Although urinary DEDTP was screened, none was detected in any of the urine samples. The presence of this breakdown product would have indicated potential exposure to other organophosphate compounds during the study period.

Worker 0030 had an elevated level of DETP on day 0 (pre-application) and day 3 that may indicate a supplemental exposure to an organophosphate or its metabolites. The absence of similarly elevated levels of DEP on day 0 and day 3 for this worker implies that elevated DETP levels may be due to dietary factors (CDC 2003). The diet of this individual varied substantially from that of the other workers due to allergies to beef and pork, and consisted principally of Asian-style vegetable dishes. Vegetables previously exposed to an organophosphate insecticide may contain metabolite residues (Zhang and Krieger 2004). Based on the relatively high level of DETP in the absence of similarly high levels of DEP, data for worker 0030 on days 0 and 3 were excluded as outliers from all statistical analyses.

The absorbed daily dose and dosage of diazinon for all workers ranged from 8 to 96 μg/day and from 0.1 to 1.6 μg/kg-day, respectively. Biomonitoring studies are especially useful to measure cumulative and aggregate exposure of compounds like diazinon that have a short metabolic half-life (metabolism and excretion in less than 48 hrs) by summing the absorbed daily dose over an appropriate number of consecutive days. The expected half-life of diazinon is less than 48 hr (EXTOXNET 1996). Thus, a sum of the five consecutive post-exposure daily dose values would be expected to account for over 97% of the total absorbed diazinon resulting from the initial exposure. In this study, aggregate exposure for each worker, measured as the sum of dose values over the 5 day sample period, ranged from 67 to 212 μg with a median dose of 103 μg.

There were no significant differences in absorbed daily dose or dosage of diazinon by day (Table 3). Thus, worker exposure to diazinon during pesticide application on day 0 was apparently too low to result in a measurable increase in diazinon metabolites for post-exposure urine samples compared to pre-exposure (control) urine samples. Given the expected dermal route of diazinon exposure and the <48 hr half-life of diazinon, it was expected that diazinon metabolites would be near their peak in day 2 and 3 post-exposure urine samples.

Table 2. Worker exposure to diazinon based upon urine biomonitoring

Time	Worker	Body Weight (kg)	Urine measurement			Diazinon exposure	
			Creatinine (g/L)	DEP (μg/L)	DETP (μg/L)	Dose (μg/day)	Dosage (μg/kg/day)
Day 0 (control)	0010	84	2.32	5.6	7.7	18	0.2
	0020	106.6	1.30	2.5*	2.5*	12	0.1
	0030	61.2	1.07	6.5	44.6†	147†	2.4†
	0040	95.3	1.34	2.5*	2.5*	12	0.1
	0050	72.6	0.85	5.5	2.5*	31	0.4
Day 1	0010	84	1.72	9.5	9.3	35	0.4
	0020	106.6	0.76	2.5*	2.5*	21	0.3
	0030	61.2	2.14	6.8	6.9	20	0.2
	0040	95.3	2.02	2.5*	2.5*	8	0.1
	0050	72.6	1.73	2.5*	2.5*	9	0.1
Day 2	0010	84	1.87	2.5*	2.5*	9	0.1
	0020	106.6	0.99	2.5*	2.5*	16	0.2
	0030	61.2	1.24	6.7	6.0	33	0.3
	0040	95.3	1.87	2.5*	2.5*	9	0.1
	0050	72.6	1.54	7.3	2.5*	21	0.2
Day 3	0010	84	0.78	2.5*	2.5*	21	0.3
	0020	106.6	0.75	6.0	2.5*	37	0.6
	0030	61.2	1.83	12.2	44.2†	96†	1.6†
	0040	95.3	3.20	7.9	2.5*	11	0.2
	0050	72.6	1.19	6.9	11.8	50	0.8
Day 4	0010	84	1.91	2.5*	2.5*	8	0.1
	0020	106.6	1.07	2.5*	2.5*	15	0.2
	0030	61.2	0.77	9.6	2.5*	52	0.5
	0040	95.3	3.89	14.5	7.4	18	0.2
	0050	72.6	1.47	16.2	2.5*	42	0.4
Day 5	0010	84	2.09	2.5*	2.5*	8	0.1
	0020	106.6	1.16	2.5*	2.5*	14	0.2
	0030	61.2	1.43	2.5*	2.5*	11	0.2
	0040	95.3	2.12	5.2	9.4	22	0.3
	0050	72.6	1.50	2.5*	2.5*	11	0.1

† Excluded as an outlier.

* Metabolite concentration was below the detection limit. For statistical purposes, a value that is half of the limit of quantification (LOQ = 5.0 μg/L) was used.

There were also no significant differences in absorbed daily dose of diazinon between individual workers (Table 4). Exposure was not greater for workers who would have been expected to have greater contact with diazinon, including workers who loaded the pump dusters, those who applied a greater amount of diazinon dust, or those who treated a greater number of rodent burrows during this

Table 3. Absorbed daily dose and dosage of diazinon for each day

Time	Mean Dose μg/day	Median Dose μg/day	Mean Dosage μg/kg-day	Median Dosage μg /kg-day
Day 0 (control)	18.3 ± 8.8	15.3	0.2 ± 0.1	0.2
Day 1	18.8 ± 11.0	20.5	0.2 ± 0.1	0.2
Day 2	17.4 ± 10.1	16.2	0.2 ± 0.2	0.2
Day 3	29.5 ± 17.3	28.8	0.3 ± 0.2	0.3
Day 4	27.1 ± 18.8	18.3	0.4 ± 0.3	0.2
Day 5	13.0 ± 5.3	11.2	0.2 ± 0.1	0.1

Row means are not significantly different (P>0.05) by Duncan's Multiple Range Test.

Table 4. Absorbed daily dose and dosage of diazinon for each worker

Time	Worker	Mean Dose μg/day	Median Dose μg/day	Mean Dosage μg/kg-day	Median Dosage μg/kg-day
Day 0 (control)	All 5 workers	18.3 ± 8.8	15.3	0.2 ± 0.1	0.2
Day 1 to Day 5	0010	16.0 ± 11.9 [a]	8.6	0.2 ± 0.1 [b]	0.1
	0020	20.6 ± 9.6 [a]	16.2	0.2 ± 0.1 [b]	0.2
	0030	29.1 ± 17.5 [a]	26.7	0.5 ± 0.3 [a]	0.4
	0040	13.4 ± 6.2 [a]	10.7	0.1 ± 0.1 [b]	0.1
	0050	26.5 ± 18.5 [a]	20.9	0.4 ± 0.3 [a,b]	0.3
	All 5 workers	20.8 ± 13.5	17.2	0.3 ± 0.2	0.2

Row means followed by the same letter are not significantly different (P>0.05) by Duncan's Multiple Range Test.

study. Elevated exposure is likely to be a product more of stochastic exposure events than of cumulative time of contact with a pesticide. Such stochastic events might include breach of protective clothing due to tears or rips, or other unintended dermal, mucosal, or inhalation pesticide contact. Our results suggest that no such unexpected exposure events occurred to any of the workers during this study. In contrast, there were significant differences in absorbed daily dosage of diazinon between individual workers (Table 4) with the worker having the lowest body weight (worker 0030) also having a significantly higher mean absorbed daily dosage.

Table 5. Human exposures (dosage) and response to diazinon

Exposure Scenario	Response	diazinon (μg/kg-day)
LOAEL[a]	Decrease in Plasma ChE Activity	20 – 30[c]
NOAEL[b]	No Adverse Effect	< 20[c]
Occupational	No Adverse Effect	14[d]
ADI[e]	No Adverse Effect	2[e]
Workers (this study)	No Adverse Effect	0.3
General Population	No Adverse Effect	0.01[f]

a: LOAEL, Lowest Observed Adverse Effect Level.
b: NOAEL, No Observed Adverse Effect Level.
c: Hayes, Pesticides studied in man, 1982.
d: Allowed occupational exposure (TLVs® and BEIs® 2000). Cutaneous exposure by contact with chemical vapors or, of probable greater significance, by direct skin contact with the substance including exposure at mucous membranes or the eyes, can significantly contribute to overall exposure. Dosage was calculated based on an occupational air concentration of 0.1 mg/m^3. Breathing rate of 10 m^3/ 8 hr and 70 kg body weight were assumed.
e: ADI, Acceptable Daily Intake. From exposure guidelines on EXTOXNET web site (http://ace.ace.orst.edu/info/extoxnet/pips/diazinon.htm).
f: CDC, Second National Report on Human Exposure to Environmental Chemicals, 2003. Dosage of diazinon was calculated based on urinary concentration of diethylphosphates (DEP, DETP and 3,5,6-TCP) by subtracting the concentration of TCP, which is the specific biomarker of chlorpyriphos. The difference is attributed to dietary diazinon exposure. It is assumed that diethylphosphates result from exposure to the two most common pesticides producing DEP and DETP metabolites (diazinon and chlorpyriphos).

In 2003, the Centers for Disease Control (CDC) released the Second National Report on Human Exposure to Environmental Chemicals (CDC 2003) in which measurement of urinary diethylphosphates was reported for the general population. The results showed that median levels of DEP and DETP for the general adult population were 1.0 and 0.48 μg/L, respectively, giving an absorbed daily dosage value for diazinon of 0.01 μg/kg-day (Table 5). In our study, pre-exposure (day 0) median absorbed daily dosage was 0.2 μg/kg-day, indicating that workers had a higher background exposure to diazinon than the general population, perhaps due to pre-application contact with diazinon during pesticide transport to the study site. The source of exposure for workers in this study is expected to be primarily dermal (and limited respiratory) rather than dietary as would be expected for the general population. The highest median absorbed daily dosage value (on day 3 post-exposure) during this study was 0.3 μg/kg-day. Exposure of workers in this study was well below the Lowest Observed Adverse

Effect Level (LOAEL) for diazinon of 20-30 μg/kg-day (Hayes 1982), as well as below the allowed occupational exposure level of 14 μg/kg-day (TLVs and BEIs 2000) and the Acceptable Daily Intake (ADI) level of 2 μg/kg-day, and thus does not present a health concern.

It is critical to continue these studies in order to provide the USEPA with human exposure data for the pesticides and application techniques used in the control of public health pests. Lacking this information, the USEPA has no alternative but to determine exposure and risk using very conservative exposure estimates or human exposure data acquired from the agricultural use of these pesticides. Pesticides used in public health pest control are generally applied in more targeted control operations, in smaller quantities, and at lower concentrations of active ingredient than would be true for their use in agriculture.

This study provides evidence that the use of 2% diazinon dust for the control of rodent fleas is safe and does not pose a health risk to workers when applied by trained professional public health pesticide applicators.

REFERENCES

Barnes AM (1982) Surveillance and control of bubonic plague in the United States. Symp Zool Soc London 50: 237-270

CDC (2003) Second national report on human exposure to environmental chemicals. Centers for Disease Control and Prevention, Atlanta

Cody RP, Smith JK (1997) Applied statistics and the SAS programming language, 4th ed. pp. 182-186. Prentice Hall, Upper Saddle River, NJ

EXTOXNET (1996) Extension Toxicology Network: Pesticide Information Profiles. http://ace.ace.orst.edu/info/extoxnet/pips/diazinon.htm

Hayes WJ (1982) Pesticides studied in man. p387. Williams & Wilkins, Baltimore

Krieger RI (2001) Handbook of pesticide toxicology, 2nd ed. pp. 929-931. Academic Press

Rose RI (2001) Pesticides and public health: Integrated methods of mosquito management. Emerg Infect Dis 7: 17-23

Smith CR (2002) Surveillance and control. *In*: California Compendium of Plague Control. pp. 8-12. California Department of Health Services, Vector-Borne Disease Section, Sacramento, CA

Snyder WS (1994) Report of the task group on reference man. International Commission on Radiological Protection (ICRP) Publication 23, p355. Tarrytown, NY

TLVs® and BEIs® (2000) Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists (ACGIH®) p29

Zhang X, Krieger RI (2004) Dialkyl phosphates (DAP) in produce confound biomonitoring in organophosphate risk assessment. The Toxicologist, Vol. 78(1-S). p. 109. Society of Toxicology 43rd Annual Meeting, March 21-25, 2004. Baltimore, MD