# EVALUATION OF FLUORIDE LEVELS AND EFFECTS ON HONEY BEES (<u>Apis mellifera</u> L.) (Hymenoptera: Apidae)

by

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SUMMARY: A three year study (1984-1986) conducted in the Puyallup Valley, WA, showed that fluoride was present in live honey bees, dead honey bees, teneral adults and stored pollen. Fluoride levels depended upon the location of the colonies in relation to a fluoride emitting source, - an aluminum smelter, and prevailing wind direction. Data on frames of adult bees, frames of brood, brood survival, brood population dynamics and honey production were collected during the study. Results indicate that the levels of fluoride found were not detrimental to the colonies over the length of the study.

# KEY WORDS: Fluoride; Honey bees, Apis mellifera.

### Introduction

Honey bees, <u>Apis mellifera</u> L. are subject to diseases, pests, pesticide poisoning and industrial pollutants which may result in bee mortality and loss of colony vigor. Fluoride gases and particulates are released as by-products from aluminum smelters and other industrial factories. Airborne gaseous fluorides can affect the growth, quality, and productivity of plants and animals (1).

Reports concerning fluoride levels in honey bees and their response to airborne fluoride emission are meager. This is especially true in North America where improvements in air cleaning equipment have removed most of the fluoride emitted from smelters (2). Dewey (3) found higher fluoride concentration in honey bees collected near an aluminum factory than in bees collected at least 80 km away from the plant. Bromenshenk et al. (4) found a higher fluoride concentration in honey bees collected near an industrial area than in bees collected some kilometers away. Atkins, Anderson and Greywood (5) demonstrated that continuous exposure to fluoride gases of 4-5 ppb (3.2-4.0  $\mu$ g/cubic meter) shortens the life-span of caged worker bees.

This paper reports results of research on effects and biological responses of honey bee colonies to different levels of airborne fluoride emissions under field conditions.

#### Materials and Methods

In January 1984, four honey bee colonies were placed at each of three

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locations in and near the Puyallup Valley, Pierce County, WA selected to provide high, medium and low exposures of the bees to fluoride. Locations were separated by several kilometers at different distances and directions from the Kaiser Aluminum plant located at Fife, WA. The Fife Heights (FH) location was 0.8 km directly downwind and in the fume path from the smelter, adjacent to a station that measured among other variables, ambient air fluoride concentration. The Valley Road Farm (VRF) location was 6.4 km east of the plant in an area occasionally subjected to fumes from the smelter. The third location was 10 km south southeast of the plant at the Washington State University Research Station (REC), an area not directly subjected to fumes from the smelter. A control location was established in a non-industrial area near Prosser, WA, approximately 200 km east of the Puyallup Valley. In March 1984, all colonies were inspected and those not surviving the winter were replaced with viable colonies from outside the area. All colonies were removed from the locations in the fall of 1984. In April, 1985, four honey bee colonies established from package bees were again placed at each of the three locations and at Prosser.

Live and dead adult honey bees, teneral adults, stored pollen, and honey obtained from each colony at each location were collected and analyzed for fluoride content. Samples for fluoride analysis were collected from each colony as follows: (1) live adult bees at the hive entrance; (2) dead bees from Todd traps (6); (3) teneral adults from 50 capped cells; (4) stored pollen from 50 cells per colony by use of forceps; and (5) all the honey from one frame per colony at two different dates each year. Adult bees (200 to 400 per sample) and pollen were analyzed at Boyce Thompson Institute, Ithaca, NY by the semi-automated method for fluoride. Honey was analyzed for fluoride at the Irrigated Agriculture Research and Extension Center by the fluoride specific electrode method.

Each year, beginning in April 1984 and continuing until fall, each individual colony was inspected every 10 to 14 days. Amounts of brood were estimated in terms of number of square centimeters of brood. In 1986, only amounts of capped brood were estimated. Number of adult bees was estimated in terms of numbers of frames covered with bees. In August, honey production was evaluated in terms of number of Langstroth combs or tenths. At each inspection 100 cells containing eggs were tagged with stick pins to outline that section of the brood comb. At the next inspection, the number of cells with brood vs empty cells were counted to obtain an estimate of brood survival. Brood population dynamics were determined by removing the cell cap and recording the number of brood at different stages of growth. During July, August, and September, Todd traps were attached to the colonies for one or two weeks to obtain estimates of adult mortality. Duncan's Multiple Range Test was used to analyze data for statistical differences between locations in concentrations of fluoride and biological parameters (7).

#### Results and Discussion

<u>Fluoride Content</u>. The mean contents of fluoride in (or on) live bees, dead bees, stored pollen and honey from the four locations are presented in Tables 1, 2 and 3.

Mean fluoride concentrations of live adult bees was significantly different between all locations in 1984 (Table 1). Fluoride concentrations were highest

Volume 21, No. 3 July, 1988 Mean Fluoride Content of Live are ppm Fluoride on a Dry Weig

	Ma		
Location	1985	1986	198
REC	58'	28'	67'
VRF	731	64 <sup>2</sup>	42'
FH	244 <sup>2</sup>	123 <sup>3</sup>	149
Prosser	9 <sup>3</sup>	-	103
1 tion	S	eptembe	er
Location	1984	1985	198
REC	85.1	66.12	72
VRF	90'	118 <sup>2</sup>	21
FH	219 <sup>2</sup>	257 <sup>3</sup>	26
Prosser	5 <sup>3</sup>	164	-

\* REC = Washington State Un and FH = Fife Heights.

Means within a column referen (p ≤ 0.5; by Duncan's [1951] Mu

at the FH location and the lintermediate. Dewey (3) four 221.0 ppm in live bees Bromenshenk et al. (4) four ppm in live bees near an i Washington. In neither study, found had contributed to hon that foraging bees exposed  $\mu$ g/bee. At FH, our area of high of 263 ppm (10.5  $\mu$ g) in October. There appears to obtained from live bees dur in October. Also, this fall recentration in the air at the foraging in October results live bees.

Mean fluoride concentr between locations (Table 2). between live and dead bees city was not related to mort fluorine toxicity is 10 µg/bee bees collected from one colo centration. We suspect that of the bee rather than inside

	Table	<u>e 1</u>				
Mean Fluoride Content of Live Honey	Bees	Collected	at	Different	Locations.*	Values
are nom Eluoride on a Dry Weight Bas	IS.					

to pp			hu	ne		July			August	
Location	1985	1986	1985	1986	1984	1985	1986	1984	1985	1986
REC	581	28'	671	48.'	971	35'	66'	138'	112'	99.1
VRE	731	64 <sup>2</sup>	42'	50 <sup>1</sup>	163 <sup>2</sup>	123 <sup>2</sup>	87'	151 <sup>1</sup>	223 <sup>2</sup>	181 <sup>2</sup>
FH	244 <sup>2</sup>	123 <sup>3</sup>	149 <sup>2</sup>	128 <sup>2</sup>	129.13	<sup>2</sup> 181 <sup>3</sup>	236 <sup>2</sup>	251 <sup>2</sup>	263 <sup>2</sup>	186 <sup>2</sup>
Prosser	9 <sup>3</sup>	3	10 <sup>3</sup>	7 <sup>3</sup>	18 <sup>3</sup>	12 <sup>2</sup>	8 <sup>3</sup>	15 <sup>3</sup>	6 <sup>3</sup>	6 <sup>3</sup>
	ç	Sentembe	er		Octo	ber	in the second	Mean		of light
Location	1984	1985	1986	19	984	1986	1984	1985	1986	
REC	85'	66.12	72'	2	271	30'	861	681	571	
VRE	90'	118 <sup>2</sup>	211 <sup>2</sup>	2	281	66²	108 <sup>2</sup>	1181	110 <sup>2</sup>	
FH	219 <sup>2</sup>	257 <sup>3</sup>	261 <sup>2</sup>	8	32 <sup>2</sup>	130 <sup>3</sup>	170 <sup>3</sup>	219 <sup>2</sup>	177 <sup>3</sup>	
Prosser	5 <sup>3</sup>	164		240144		54	134	11 <sup>3</sup>	74	

\* REC = Washington State University Research Station; VRF = Valley Road Farm; and FH = Fife Heights.

Means within a column referenced by the same number are not significantly different ( $p \le 0.5$ ; by Duncan's [1951] Multiple Range Test).

at the FH location and the lowest at Prosser, with the REC and VRF locations intermediate. Dewey (3) found 10.5 ppm fluoride in control honey bees and 221.0 ppm in live bees near a fluoride emission source in Montana. Bromenshenk et al. (4) found 4 ppm fluoride in control honey bees and 182 ppm in live bees near an industrial area in the same area of Puget Sound, Washington. In neither study, was there any indication that levels of fluoride found had contributed to honey bee mortality. In Europe, Dreher (8) determined that foraging bees exposed to heavy fluorine pollution contained 9.2 to 10.5  $\mu$ g/bee. At FH, our area of highest fluoride concentration, we found a mean high of 263 ppm (10.5  $\mu$ g) in August and a mean low of 82 ppm (3.3  $\mu$ g) in October. There appears to be an increase in mean fluoride concentration in October. Also, this fall reduction is not clearly related to the fluoride concentration in the air at the site (Figure 1). We speculate that decreased pollen foraging in October results in lower concentrations in the fluoride content of live bees.

Mean fluoride concentration of dead bees was significantly different between locations (Table 2). There were no significant differences in fluoride between live and dead bees at the four locations indicating that fluoride toxicity was not related to mortality. Dreher (8) has suggested that the  $LD_{50}$  for fluorine toxicity is 10 µg/bee. We found 358 ppm (14.3 µg) fluoride from live bees collected from one colony at FH suggesting 10 µg/bee is not a toxic concentration. We suspect that much of the fluoride is on the surface of the body of the bee rather than inside it.

Fluoride



Fluoride was significantly higher only from teneral adults collected at FH in both years. Mean values of fluoride levels in teneral adults were: REC 3 ppm, VRF 3 ppm, FH 15 ppm, and Prosser 1 ppm, for 1985 and REC 8 ppm, VRF 11 ppm, and FH 17 ppm for 1986.

Fluoride in stored pollen was lowest at Prosser and highest at FH. The VRF site was significantly higher than the REC site only in 1986 (Table 3).

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Location
REC .
VRF
FH
Prosser

Means within a column fol different ( $p \le 0.5$ ; by Duncan

Mean	Fluoride	C	onte	nt
	Value	s	are	pp

	19	84
Location	Pollen	Ho
REC	16 <sup>a</sup>	1.
VRF	19 <sup>a</sup>	1.
FH	33 <sup>b</sup>	0
Prosser	-	

Means within a column fol different ( $p \le 0.5$ ; by Duncan

There have been few analyse Maurizio and Staub (9) repor collected in Switzerland.

Fluoride concentrations (10) who reported concentra New York, did not feel that of 1.4 ppm fluoride for home

No consistent patterns r of the experiment (Table 4 populations. The existing br decline in fall were normal f

Mean brood survival for 91% at FH and 91.6% at Prodetected in brood survival be presence of airborne fluorid (11) found average egg mo influenced by both colony a

#### Table 2

Mean Fluoride Content of Dead Honey Bees Collected in Todd Traps at Different Locations. Values are ppm Fluoride on a Dry Weight Basis.

Location	1984	1985
REC	102 <sup>a</sup>	74 <sup>a</sup>
VRF	144 <sup>b</sup>	130 <sup>b</sup>
FH	223 <sup>C</sup>	219 <sup>C</sup>
Prosser	15 <sup>d</sup>	

Means within a column followed by the same letter are not significantly different ( $p \le 0.5$ ; by Duncan's [1951] Multiple Range Test).

a	DI	е	3
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Mean Fluoride Content of Pollen and Honey at Different Locations. Values are ppm Fluoride on a Dry Weight Basis.

198		84	19	85	5 1986		
Location	Pollen	Honey	Pollen	Honey	Pollen	Honey	
REC	16 <sup>a</sup>	1.2 <sup>a</sup>	18 <sup>a</sup>	0.4 <sup>a</sup>	18 <sup>a</sup>	0.9 <sup>ab</sup>	
VRF	19 <sup>a</sup>	1.2 <sup>a</sup>	21 <sup>a</sup>	0.7 <sup>b</sup>	32 <sup>b</sup>	0.8 <sup>b</sup>	
FH	33 <sup>b</sup>	0.9 <sup>a</sup>	37 <sup>b</sup>	0.7 <sup>b</sup>	61 <sup>C</sup>	1.4 <sup>a</sup>	
Prosser	na <u>t p</u> a w	i mangai	8 <sup>C</sup>	0.3 <sup>a</sup>	2 <sup>d</sup>	0.4 <sup>C</sup>	

Means within a column followed by the same letter are now significantly different ( $p \le 0.5$ ; by Duncan's [1951] Multiple Range Test).

There have been few analyses of the fluoride content in stored pollen, although Maurizio and Staub (9) reported from 9 to 18 ppm fluoride in various pollens collected in Switzerland.

Fluoride concentrations in honey were always low (Table 3). Tong et al. (10) who reported concentrations of 0.001 to 8.9 ppm fluorine in honey from New York, did not feel that the high amount was hazardous. We found a high of 1.4 ppm fluoride for honey in our study.

No consistent patterns related to fluoride emerged during the three years of the experiment (Table 4) i.e. fluoride had no effect on adult or brood populations. The existing brood, and colony build up during the summer and decline in fall were normal for honey bees.

Mean brood survival for the 3 year study was 91% at REC, 90.3% at VHF, 91% at FH and 91.6% at Prosser. In 1984 and 1986 significant differences were detected in brood survival between locations, but they were not related to the presence of airborne fluoride. No differences were detected in 1985. Harbo (11) found average egg mortality of 7% and concluded that mortality was influenced by both colony and queen. Sakagami and Fukuda (12) found 4-11%

Fluoride

Table 4

Bee Populations in Colonies at Different Locations in the Puyallup Valley

Location	Mean Number of Frames per Colony Covered by Adult Bees			Mean S of E	Square Centi Brood per Co August	meters lony
Looution	1984	1985	1986	1984	1985	1986*
REC	22 <sup>a</sup>	16 <sup>a</sup>	12 <sup>a</sup>	5321 <sup>a</sup>	10217 <sup>a</sup>	3483 <sup>a</sup>
VRF	22 <sup>a</sup>	17 <sup>a</sup>	22 <sup>b</sup>	8727 <sup>ab</sup>	10210 <sup>a</sup>	5572 <sup>ab</sup>
FH	11 <sup>a</sup>	18 <sup>a</sup>	24 <sup>b</sup>	7359 <sup>ab</sup>	10681 <sup>a</sup>	6095 <sup>a</sup>

\* Mean square centimeters of capped brood per colony.

Means within a column followed by the same letter are not significantly different (p  $\leq$  0.5; by Duncan's [1951] Multiple Range Test.

mortality to the capped cell stage in normal, healthy colonies, about the same as that we found.

Stage of brood development 14 days after eggs were marked is presented in Figure 2. No consistent differences were evident between locations or time of year of sampling in the percent of individuals at the different stages of development. Milum (13) showed that variations for complete development times of individual worker bees ranged from slightly less than 19  $^{7}/_{8}$  days to slightly more than 24 days and developmental time of a population approached a bell-shaped curve. Our data show a similar curve for an immature population of honey bees.

The mean number of dead bees per day ranged from 6 to 85, but there were no significant differences between locations. The number of dead bees were in the normal range of less than 100 per day at all locations (14).

There were no significant differences between locations in honey production in 1984 (Table 5); there were, however, in 1985 and 1986. Woyke (15) showed that honey production is correlated to a varying degree with worker population and average length of productive life of workers.

Toble F

	140		
Mean Frames o	f Honey in A	ugust at Diffe	erent Locations
Location	1984	1985	1986
REC	7"5 <sup>a</sup>	2.8 <sup>a</sup>	2.3 <sup>a</sup>
VRF	10.6 <sup>a</sup>	1.5 <sup>a</sup>	2.2 <sup>a</sup>
FH	9.2 <sup>a</sup>	5.9 <sup>b</sup>	7.3 <sup>b</sup>
Prosser	i ba <u>n</u> ingali	9.3 <sup>C</sup>	11 C

Means within a column followed by the same letter are not significantly different ( $p \le 0.5$ ; by Duncan's [1951] Multiple Range Test.

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Fluoride

#### Mayer, Lunden and Weinstein

#### Conclusion

Since in our study honey production was equal to or higher at FH than at the other locations in the Puyallup Valley, the higher concentrations of fluoride appeared to have no effect on the size of worker populations or adult life span.

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M.W. Bilbeissi, C. Frays

SUMMARY: A total in a chronic dental Fluorosis and daily for each child. Drin collected and analyze by each child was dental fluorosis and positive.

**KEY WORDS:** Dental fluorosis

Epidemiological studies fluoride in drinking water a during recent decades (1-13) are found at the foot of hig of marine origin. The geograp and the Rift Valley through originate from volcanic activ industrial emissions and from

Epidemiological studies (16,8,13) showed an unexper fluorosis in populations livin drinking water. Reports to t viously indicated that fluori 0.40 ppm F to 1 ppm F in phosphate mines where it was

The purpose of our stu fluorosis and the source of hi

2,516 children (1,180 g examined in 26 schools in 1 iners. Frequent inter- and the survey.

Schools were chosen ra in advance time or date of had official permission to v

Unité de Recherche LN de Chirurgie Dentaire, 01, France.