# FENITROTHION RESIDUES IN HONEYBEES AND THEIR PRODUCTS COLLECTED FROM FORESTED AREAS SPRAYED WITH THE INSECTICIDE FOR BUDWORM CONTROL

By

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# INTRODUCTION

Fenitrothion [0,0-dimethyl-0-(4-nitro-m-totyl) phosphorothioate] is a widely used organophosphorus pesticide because of its low mammalian toxicity and biological selectivity. It has become the major replacement for DDT in protecting Canadian forests against the ravages of spruce budworm. Application of the insecticide by aircraft at an operational dosage of 0.14 to 0.28 kg/ha (2 to 4 oz A.I./acre) in spring, prevented defoliation by the insect pest, without causing serious ecological damage (Buckner 1974). Because of its wide usage in forestry since 1968 (Fettes 1968), extensive research has been stimulated concerning its effect on various nontarget species of fauna inhabiting the forest and exposed to the toxicant. In recent years the loss of honeybees (*Apis mellifera* L.) due to insecticides applied for forest pest control has become a serious problem (Atkins *et al* 1970). Information on the effect and hazards of fenitrothion to honeybees applied under operational conditions for budworm control are not readily available. This report embodies the results and observations made in an extensive three-year study program undertaken in 1972 to determine the effect of fenitrothion applied by aircraft at operational levels on honeybees and its residues, if any, in 122 honeybee, pollen, beeswax and honey samples collected from sprayed forest areas in the provinces of Ontario and Quebec.

## MATERIALS AND METHODS

### Honeybees and their Products

The honeybees and their products used in the present investigation were collected from hives kept in forest areas of Ontario and Quebec sprayed with fenitrothion and supplied by C.H. Buckner and B.B. McLeod of this Institute.

### Analytical Methods

Fenitrothion residues, its oxygen analog and its cresol hydrolysis product were determined in the collected samples according to the analytical methodology recently developed at this Institute (Sundaram 1974a). The essential steps involved are the extraction of the substrates with acetonitrile followed by hexane partition and charcoal-Celite column cleanup. The parent compound and the metabolites were separated by differential elution using a deactivated silica-gel column and the fenitrothion and the oxon analysed by gas-liquid chromatography (GLC) with a flame photometric detector sensitive to phosphorus and the cresol with a Ni-63 electron capture detector. The volumes of acetonitrile extractant used were adjusted according to the mass of the bees and their products used in the analysis.

## RESULTS AND DISCUSSION

Residues of fenitrothion and its metabolites found in the various bee samples and their products collected from control and sprayed sampling sites in Ontario and Quebec since 1972 are given in Tables 1 to 17. The concentration of the samples is expressed in units of ppm "as sampled" under forest conditions including moisture content etc. as variables.

Bee samples collected from Lamacaza (P.Q.), soon after the first application of fenitrothion in early May 1974 (Table 1), did not contain detectable levels (> 0.02 ppm) of the insecticide but after an interval of 2 days (48 hrs), the dead bees showed a level of 0.04 ppm of fenitrothion. After the second application in early June, the bees sampled 27 hours later, showed a maximum of 0.42 ppm which dissipated rapidly to 0.04 ppm within two days. Not all the samples analysed contained detectable levels of fenitrothion and none of them had any oxon or the cresol metabolite. Some of the live bee samples collected at different intervals of time after the two spray applications contained 0.08 to 0.02 ppm of fenitrothion. The single bee sample received from Dieppe, N.B. contained 0.13 ppm of fenitrothion indicating that the bees were exposed to the insecticide spray. No fenitrothion and its oxygen analog were found in the Shawville (P.Q.) bee samples (Table 3); it is likely that these samples were collected from sites which were relatively isolated from insecticide application.

Among the bee (Table 4) and pollen (Table 5) samples analysed during the 1973 summer spray program at Larose Forest, only one bee sample out of the six analysed contained fenitrothion (0.03 ppm); others did not contain either the toxicant or its oxidation product in measurable amounts. Six out of the nine pollen samples collected from the hives kept in the sprayed area contained low but detectable amounts of fenitrothion ranging from 0.044 to 0.001 ppm.

An indepth and extensive study on the residue levels and persistence of fenitrothion in honeybees and their products was carried out during the 1972 summer spray program at Larose Forest and the results are recorded in Tables 6 to 17. An examination of the data shows that none of the prespray samples of bees (Tables 6 and 7), pollen (Tables 9 and 10), honey (Tables 12 and 13) and beeswax (Tables 15 and 16) contained any detectable amounts of fenitrothion residues. The check samples of bees and pollen collected from the control hive (CH) at the intervals of zero to two days (Tables 8 and 11) after the treatment, contained negligible amounts (0.005 ppm to traces, i.e. < 0.005 ppm) of fenitrothion. Samples of bees and their products collected from hives kept in the sprayed plot BR (Bee Range) 86 and 224 contained only the parent compound, the oxon and cresol metabolites were not detectable in any of the samples studied.

The initial fenitrothion concentration of the bee samples collected on day zero from the hive BR-86 was 2.080 ppm compared to the 0.405 ppm in bees from hive BR-224. Generally the insecticide levels were low in hive BR-224 compared to hive BR-86. The results in

Table 6 and 7 show that the dissipation rate of the insecticide in both hives was high. This indicates that the half-life of the insecticide in bees was short (Ibrahim et al 1971).

The recoveries of fenitrothion residues from pollen (Tables 9 and 10) showed only the parent compound detectable; no oxon nor cresol were found. The residue levels in pollen were comparatively high in hive BR-224 showing a maximum amount of 0.175 ppm on the fifth day and persisting in near trace amounts (0.010 ppm) up to 29 days whereas in hive BR-86, the highest concentration was 0.015 ppm observed on the first day which disappeared within a week. Trace amounts of fenitrothion were found in the pollen samples collected from the control hive (Table II).

Prespray samples of honey collected from all three hives (BR-86, BR-224 and CH) did not contain any fenitrothion whereas the postspray samples collected 49 days after treatment from BR-86 and 29 days from BR-224 contained only traces (< 0.005 ppm) of the toxicant (Tables 12-14).

The postspray samples of wax collected from the hives in the same periods as the honey, contained low (0.055 ppm in BR-224 and 0.005 ppm in BR-86) but detectable amounts of fenitrothion. Relatively low amount of the insecticide was (0.005 ppm) found in hive BR-86 after the long interval (49 days) of post-treatment.

The data presented in this report show that fenitrothion was short-lived and rapidly degraded in bees. The toxic oxon metabolite and the hydrolytic product, 3-methyl-4-nitrophenol, were not found in bees and their products investigated. It is likely, as observed in

other insects in presence of similar organophosphorus insecticides (Lewis 1969, Lewis and Lord 1969, Miyamoto 1969, O'Brien 1960, 1967), that honeybees probably detoxify fenitrothion rapidly by hydrolytic routes to desmethyl derivatives (demethylation by phosphatase enzymes) yielding phosphorothioic acids as the terminal product instead of the anticipated oxidative desulfuration mechanism forming the oxon intermediate. The formation of oxon metabolite in biological systems is controlled by the presence and ready availability of reduced nicotine adenine dinucleotide phosphate ($NADPH_2$) which may be lacking in honeybees. In addition to metabolic degradation, the rapid loss of fenitrothion in bees may have been due to various physical factors such as volatilization, weathering, leaching and photolysis as observed in foliage (Yule and Duffy 1972, Sundaram 1974b, 1974c, 1975). The dissipation and metabolic breakdown of fenitrothion in honeybees is still obscure and requires some form of explorative research to be done.

TABLE 1

Fenitrothion Residues in Honeybee Samples Collected from+ Lamacaza (P.Q.)

After the First Aerial Spray - May* 1974

| Sample No. | Hours Relative to Application | Mass (g) | Fenitrothion Residues (ppm - as sampled) | | |
|---|---|---|---|---|---|
| | | | Fenitrothion | Fenitro-oxon | Cresol++ |
| 1 | Pre-spray plot(260-1) | 10.2 | N.D. | N.D. | N.D. |
| 2 | 12 | 4.1 | N.D. | N.D. | N.D. |
| 3 | 15 | 3.2 | N.D. | N.D. | N.D. |
| 4 | 18 | 2.9 | N.D. | N.D. | N.D. |
| 5 | 24 | 4.7 | N.D. | N.D. | N.D. |
| 6 | 48 | 2.1 | 0.04 | N.D. | N.D. |
| 7 | 48** | 1.9 | N.D. | N.D. | N.D. |
| 8 | 72 | 2.9 | N.D. | N.D. | N.D. |
| 9 | 72** | 1.6 | N.D. | N.D. | N.D. |
| 10 | 96 | 2.2 | N.D. | N.D. | N.D. |
| 11 | 96** | 1.9 | N.D. | N.D. | N.D. |
| 12 | 168 | 2.8 | 0.03 | N.D. | N.D. |
| 13 | 168** | 1.4 | 0.02 | N.D. | N.D. |
| 14 | 192 | 2.3 | N.D. | N.D. | N.D. |
| 15 | 216 | 4.0 | N.D. | N.D. | N.D. |
| 16 | 264 | 2.9 | T | N.D. | N.D. |
| 17 | 288 | 3.2 | N.D. | N.D. | N.D. |

* Spray Date: May 11, 1974 at 1930 hrs
(Formulation: Emulsion, 2 oz A.I./acre

** Live Bee Samples

++ 4-Nitro-m -cresol

+ Plot 260-3

N.D. Not detected

T Traces (< 0.02 ppm)

TABLE 2

Fenitrothion Residues in Honeybee Samples Collected From Lamacaza (P.Q.)

After the Second Aerial Spray - June* 1974

| Sample No. | Hours Relative to Application | Mass (g) | Fenitrothion Residues (ppm - as sampled) | | |
|---|---|---|---|---|---|
| | | | Fenitrothion | Fenitro-oxon | Cresol |
| 18 | Pre-spray | 2.7 | N.D. | N.D. | N.D. |
| 19 | 24 | 4.2 | 0.20 | N.D. | N.D. |
| 20 | 24** | 3.6 | 0.08 | N.D. | N.D. |
| 21 | 27 | 2.9 | 0.42 | N.D. | N.D. |
| 22 | 27** | 3.0 | 0.02 | N.D. | N.D. |
| 23 | 31 | 2.2 | 0.14 | N.D. | N.D. |
| 24 | 31** | 3.3 | 0.02 | N.D. | N.D. |
| 26 | 48 | 2.7 | 0.04 | N.D. | N.D. |
| 27 | 48** | 1.7 | N.D. | N.D. | N.D. |
| 28 | 120 | 4.9 | T | N.D. | N.D. |
| 29 | Post-spray from Dieppe, N.B. (June 4, 1974 - G. Landry) | 22.8 | 0.13 | N.D. | N.D. |

* Spray Date: June 6, 1974 at 2000 hrs
(Formlation : Emulsion, 2 oz A.I./acre

For other explanations, see footnotes in Table 1.

TABLE 3

Fenitrothion Residues in

Honeybee Samples Collected From Shawville, (P.Q.) - Summer 1974

| Sample No. | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) |
|---|---|---|---|
| 30 | 0.72 | N.D. | N.D. |
| 31 | 0.69 | N.D | N.D. |
| 32 | 0.70 | N.D. | N.D. |
| 33 | 0.67 | N.D. | N.D. |
| 34 | 0.75 | N.D. | N.D. |
| 35 | 0.65 | N.D. | N.D. |
| 36 | 0.61 | N.D. | N.D. |
| 37* | 1.40 | N.D. | N.D. |

N.D. Not detected

* Control samples from LaRose Forest
Minimum detectable limits: Fenitrothion 0.08 ppm
Fenitro-oxon 0.03 ppm

TABLE 4

Fenitrothion Residues in Honeybee Samples

Collected from LaRose Forest - Summer, 1973

| Sample No. | Identification No. | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) |
|---|---|---|---|---|
| 38 | BR-H5 June 12 | 10 | N.D. | N.D. |
| 39 | BR-H5 June 15 | 10 | 0.03 | N.D. |
| 40 | BR-H5 June 22 | 10 | N.D. | N.D. |
| 41 | BR-H5 June 26* | - | - | - |
| 42 | BR-H5 June 12,C | 10 | N.D. | N.D. |
| 43 | BR-H5 June 15,C | 10 | N.D. | N.D. |
| 44 | BR-H5 June 22,C | 10 | N.D. | N.D. |
| 45 | BR-H5 June 26,C* | - | - | - |

Abbreviations: BR - Bee Range
H - Hive
C - Control Plot

* Bee samples were unavailable for analysis

See also the footnotes in Table 1.

TABLE 5

Fenitrothion Residues in Pollen Samples Collected

From LaRose Forest During the Summer of 1973

| Sample No. | Identification No. | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) |
|---|---|---|---|---|
| 46 | BR-H5 June 10 | 10 | 0.001 | N.D. |
| 47 | BR-H5 June 14 | 10 | 0.004 | N.D. |
| 48 | BR-H5 June 18 | 10 | 0.012 | N.D. |
| 49 | BR-H5 June 22 | 10 | 0.010 | N.D. |
| 50 | BR-H5 June 26* | -- | -- | -- |
| 51 | BR-H5 June 10 C | 10 | N.D. | N.D. |
| 52 | BR-H5 June 14 C | 10 | N.D. | N.D. |
| 53 | BR-H5 June 18 C | 10 | 0.044 | N.D. |
| 54 | BR-H5 June 22 C | 10 | 0.005 | N.D. |
| 55 | BR-H5 June 26 C | 10 | N.D. | N.D. |

See footnotes in Tables 1 and 4

Minimum detectable limits: Fenitrothion 0.001 ppm
Fenitro-oxon 0.004 ppm

* Pollen sample was not available for analysis.

TABLE 6

Fenitrothion Residues in Honeybee Samples Collected From the Hive BR 86 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Dead Bee Count | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol+ (ppm) |
|---|---|---|---|---|---|---|
| 56 | -1** | 2 | 0.12 | N.D. | N.D. | N.D. |
| 57 | 0 | 100 | 5.38 | 2.080 | N.D. | N.D. |
| 58 | 1 | 319 | 20.39 | 0.630 | N.D. | N.D. |
| 59 | 2 | 323 | 21.29 | 0.370 | N.D. | N.D. |
| 60 | 3 | 145 | 9.02 | 0.635 | N.D. | N.D. |
| 61 | 4 | 106 | 8.00 | 0.275 | N.D. | N.D. |
| 62 | 5 | 71 | 4.84 | 0.130 | N.D. | N.D. |
| 63 | 7 | 4 | 0.25 | T | N.D. | N.D. |
| 64 | 12 | 4 | 0.32 | 0.010 | N.D. | N.D. |
| 65 | 20 | 33 | 2.95 | N.D. | N.D. | N.D. |
| 66 | 27 | 13 | 0.86 | N.D. | N.D. | N.D. |
| 67 | 49 | 7 | 0.53 | N.D. | N.D. | N.D. |

* Concn. is expressed in ppm "as sampled".
** Prespray samples
\+ 4-Nitro-m-cresol

B.R. Bee Range
T Traces (< 0.005 ppm)
N.D. Not detected.

TABLE 7

Fenitrothion Residues in Honeybees Collected from the Hive BR 224 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Dead Bee Count | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|---|
| 68 | -1 | 9 | 0.61 | N.D. | N.D. | N.D. |
| 69 | 0 | 221 | 10.74 | 0.405 | N.D. | N.D. |
| 70 | 1 | 110 | 5.62 | 0.250 | N.D. | N.D. |
| 71 | 2 | 24 | 1.98 | 0.070 | N.D. | N.D. |
| 72 | 3 | 29 | 2.39 | 0.060 | N.D. | N.D. |
| 73 | 4 | 9 | 0.61 | 0.040 | N.D. | N.D. |
| 74 | 7 | 2 | 0.10 | 0.010 | N.D. | N.D. |
| 75 | 9 | 2 | 0.12 | N.D. | N.D. | N.D. |
| 76 | 13 | 8 | 0.50 | N.D. | N.D. | N.D. |
| 77 | 19 | 5 | 0.26 | N.D. | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 8

Fenitrothion Residues in Honeybees Collected From the Control Hive in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Dead Bee Count | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|---|
| 78 | -1 | 4 | 0.21 | N.D. | N.D. | N.D. |
| 79 | 0 | 8 | 0.38 | T | N.D. | N.D. |
| 80 | 1 | 10 | 0.45 | T | N.D. | N.D. |
| 81 | 2 | 9 | 0.40 | N.D. | N.D. | N.D. |
| 82 | 4 | 6 | 0.30 | N.D. | N.D. | N.D. |
| 83 | 5 | 7 | 0.32 | N.D. | N.D. | N.D. |
| 84 | 7 | 4 | 0.18 | N.D. | N.D. | N.D. |
| 85 | 12 | 5 | 0.21 | N.D. | N.D. | N.D. |
| 86 | 20 | 7 | 0.33 | N.D. | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 9

Fenitrothion Residues in Pollen Samples Collected From the Hive BR 86 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 87 | -1 | 10.0 | N.D. | N.D. | N.D. |
| 88 | 0 | 10.0 | 0.005 | N.D. | N.D. |
| 89 | 1 | 2.0 | 0.015 | N.D. | N.D. |
| 90 | 2 | 2.0 | 0.010 | N.D. | N.D. |
| 91 | 4 | 4.1 | 0.010 | N.D. | N.D. |
| 92 | 5 | 5.0 | 0.005 | N.D. | N.D. |
| 93 | 7 | 10.0 | 0.005 | N.D. | N.D. |
| 94 | 12 | 3.5 | T | N.D. | N.D. |
| 95 | 20 | 10.0 | N.D. | N.D. | N.D. |
| 96 | 27 | 10.0 | N.D. | N.D. | N.D. |
| 97 | 49 | 5.0 | N.D. | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 10

Fenitrothion Residues in Pollen Samples Collected From the Hive BR 224 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 98 | -1 | 2.0 | T | | |
| 99 | 0 | 1.9 | 0.005 | N.D. | N.D. |
| 100 | 2 | 2.6 | 0.090 | N.D. | N.D. |
| 101 | 5 | 2.0 | 0.175 | N.D. | N.D. |
| 102 | 6 | 1.9 | 0.100 | N.D. | N.D. |
| 103 | 7 | 2.0 | 0.085 | N.D. | N.D. |
| 104 | 29 | 5.0 | 0.010 | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 11

Fenitrothion Residues in Pollen Samples Collected From the Control Hive in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 105 | -1 | 2.5 | N.D. | N.D. | N.D. |
| 106 | 0 | 1.1 | N.D. | N.D. | N.D. |
| 107 | 1 | 3.1 | T | N.D. | N.D. |
| 108 | 2 | 2.1 | 0.005 | N.D. | N.D. |
| 109 | 6 | 1.6 | N.D. | N.D. | N.D. |
| 110 | 20 | 5.0 | N.D. | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 12

Fenitrothion Residues in Honey Samples Collected from the Hive BR 86 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 111 | Pre-spray | 10.5 | N.D. | N.D. | N.D. |
| 112 | 49 | 17.8 | T | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 13

Fenitrothion Residues in Honey Samples Collected from the

Hive BR 224 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 113 | Pre-spray | 12.2 | N.D. | N.D. | N.D. |
| 114 | 29 | 9.7 | T | N.D. | N.D. |

See the foot notes in Table 6.

TABLE 14

Fenitrothion Residues in Honey Samples Collected From the Control Hive in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 115 | Pre-spray | 8.1 | N.D. | N.D. | N.D. |
| 116 | 49 | 4.2 | N.D. | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 15

Fenitrothion Residues in Beeswax Collected From the

Hive BR 86 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 117 | Pre-spray | 8.9 | N.D. | N.D. | N.D. |
| 118 | 49 | 10.8 | 0.005 | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 16

Fenitrothion Residues in Beeswax Collected From the Hive BR 224 in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 119 | Pre-spray | 7.1 | N.D. | N.D. | N.D. |
| 120 | 29 | 4.8 | 0.055 | N.D. | N.D. |

See the footnotes in Table 6.

TABLE 17

Fenitrothion Residues in Beeswax Collected from the

Control Hive in LaRose Forest - Summer 1972

| Sample No. | Days After Treatment | Mass (g) | Fenitrothion (ppm) | Fenitro-oxon (ppm) | Cresol (ppm) |
|---|---|---|---|---|---|
| 121 | Pre-spray | 4.8 | N.D. | N.D. | N.D. |
| 122 | 49 | 7.2 | N.D. | N.D. | N.D. |

See the foot notes in Table 6.

SUMMARY

Bee, pollen, honey and wax samples were collected from forest areas sprayed with fenitrothion for budworm control at the operational dosage of 2 to 4 oz A.I./acre for a three-year period since 1972. Residues of the insecticide, its oxygen analog and its cresol hydrolysis product were determined in the collected samples after extraction with acetonitrile followed by hexane partition and charcoal-Celite column cleanup. The parent compound and the metabolites were separated by differential elution using a deactivated silica-gel column and the fenitrothion and the oxon, determined by GLC with a flame photometric detector sensitive to phosphorus and the cresol with a Ni 63 electron-capture detector. No oxon or cresol derivatives of the parent compound were present in any of the substrate samples analysed with the method allowing measurement of residues as low as 0.005 ppm. The level of fenitrothion in bees was not high (Maximum 2.080 ppm) and decreased rapidly to traces within a week. Fenitrothion levels in pollen were very small and declined less rapidly than in bees. Accumulation of the toxicant in honey and wax samples were negligibly small, but traces of the insecticide seems to persist in wax for some time. The results indicate that the insecticide is lost rapidly from bee samples primarily by physical means and to a lesser extent by chemical and bio-degradation.

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