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# *Re-evaluation of the toxicity of CCME hydrocarbon Fraction 3—Year 2: Toxicity and uptake of single and binary combinations of fractions*

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# *Year 1 Overview*

- Compared toxicity of two sub-fractions of CCME Fraction 3 (F3a >$n$C$_{16}$-$n$C$_{23}$; F3b >$n$C$_{23}$-$n$C$_{34}$) to earthworms, springtails, and plants.
- Difference in definitive/chronic toxicity not sufficient to warrant regulating on sub-fractions at this time.

# *Year 2 overview*

1) Toxicity of binary combinations of fractions 2, 3a and 3b to earthworms.
2) Uptake and elimination of single and binary combinations of fractions 2, 3a and 3b by earthworms over time.
3) Repeat toxicity tests with earthworms, using new method for chemical analysis.

# *Oil fractionation*

- Light to medium Alberta crude (Federated Pipelines)
- Fractionated using ASTM methods D2892 and D5236

F2: >*n* $C_{10}$-*n* $C_{16}$

F3: >*n* $C_{16}$-*n* $C_{34}$

*F3a: >n $C_{16}$-n $C_{23}$*

*F3b: >n $C_{23}$-n $C_{34}$*

F4: >*n* $C_{34}$

# *Composition of each fraction*

## Percent Overlap Between Fractions

| Distillate | $<C_{10}$ | $>C_{10}$-$C_{16}$ | $>C_{16}$-$C_{23}$ | $>C_{23}$-$C_{34}$ | $>C_{34}$ |
|---|---|---|---|---|---|
| **F2** | 4.1% | **87%** | 8.6% | 0% | 0% |
| **F3a** | 0% | 1.4 % | **87.5%** | 11.1% | 0% |
| **F3b** | 0% | 0% | 2.1% | **90.1%** | 7.8% |
| **F3** | 0% | 0.7% | **96.3%** | | 3% |
| **F4** | 0% | 0% | 0% | 40% | **60%** |

Table values from Environment Canada (by weight)

F3: 61.6% F3a, 38.4% F3b (by weight) (Imperial Oil Ltd.)

# *Earthworm toxicity tests with binary combinations of fractions: why binary?*

- Petroleum contamination not found as discreet “fractions” but as mixtures of fractions
- Previous data indicated the toxicity of combinations of fractions was not additive
- Might have implications for regulations
- Response might be species-dependent —chose earthworms as test species

# *Earthworm toxicity tests: general method*

- *Eisenia andrei*
- 500-ml mason jar with aluminum foil lid
- 280-300 g soil w.w. (Black Chernozem)
- 24-hr light
- 22°C
- 28-d survival (54-d for F4)

# *Binary tests*

- Tested the following two combinations based on acute toxicity:

  F2F3a  F3aF3b

- Did not test combinations with F4 since not acutely toxic

# *Binary tests*

## Toxic Units

- A method for expressing toxicity of mixtures
  - Assumes concentration addition
  - Assumes similar mechanism of action

**Assumption:** Petroleum hydrocarbons thought to act by a common mechanism of action—narcosis—and therefore assumed to be concentration additive

# *Binary tests*

## Toxic Units

- For F2F3a: expressed concentrations of mixture components as fractions of their LC50
- Therefore, for binary combinations:

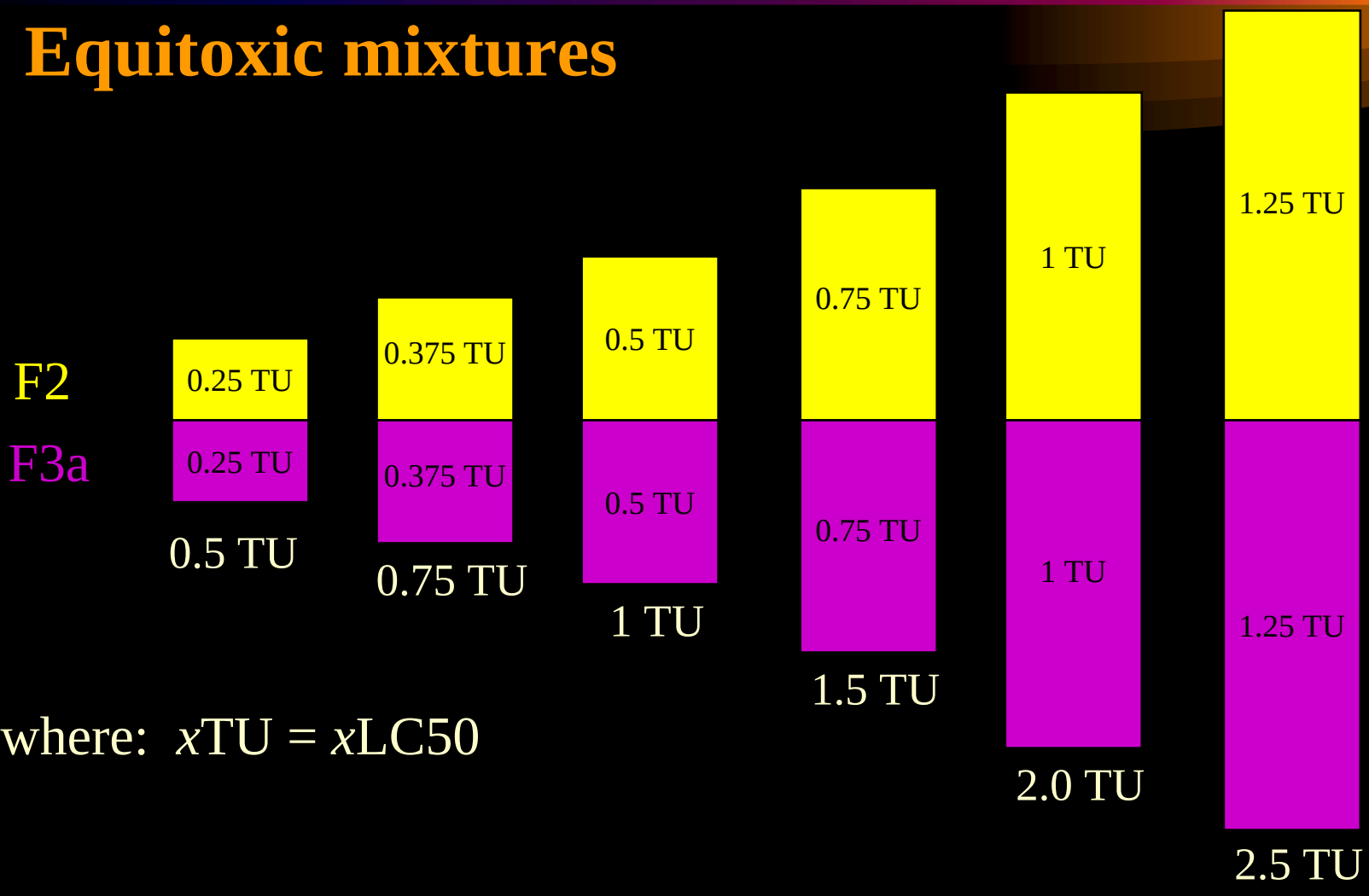
$$TU = (x_1/LC50_1) + (x_2/LC50_2)$$

where $x_i$ is the concentration of component *i*

- Used equitoxic mixtures

# *Binary tests*

## Equitoxic mixtures




where: $x$TU = $x$LC50

# *Binary tests*

- If  LC50 = 1 TU
  - Concentration additive
- If LC50 < 1 TU
  - More-than-concentration additive (synergistic)
- If LC50 >1 TU
  - Less-than-concentration additive (antagonistic)

# *Binary tests*

- For F3aF3b
  - Since F3b non-toxic, kept concentration of F3b constant (10 mg/g) and increased the concentration of F3a
  - F3a concentration corresponded to 0.5, 0.75, 1, 1.5, 2 and 2.5 TU

# *Binary tests*

## Results

F2 $LC50_{alone}$ = 0.68 mg/g;  F3a $LC50_{alone}$ = 3.5 mg/g

- F2F3a 28-d LC50 = 2.3 TU *(0.78 mg/g F2; 4.0 mg/g F3a)*
- F3aF3b 28-d LC50 > 2.2 TU *(7.5 mg/g F3a, 10 mg/g F3b)*

Therefore, the fractions interacted in a *less-than-additive* manner

# *Binary tests*

- Why less-than-additive?
  - Less bioavailable?
  - Temporal separation in exposure to the two fractions?
  - Interaction within organism?
- Uptake tests will help interpret results

# *Uptake and elimination of fractions by earthworms*

# *Uptake and elimination of fractions*

- Single:  F2, F3a, F3b
- Binary:  F2F3a, F3aF3b
- Concentration:  ½ LC50 (F2 & F3a); 10 mg/g F3b
- Uptake duration
  - F2:  16 day, transfer worms to clean soil on day 8
  - F3a & F3b:  64 days, transfer worms to clean soil on day 16
- Elimination duration
  - 8 days in clean soil

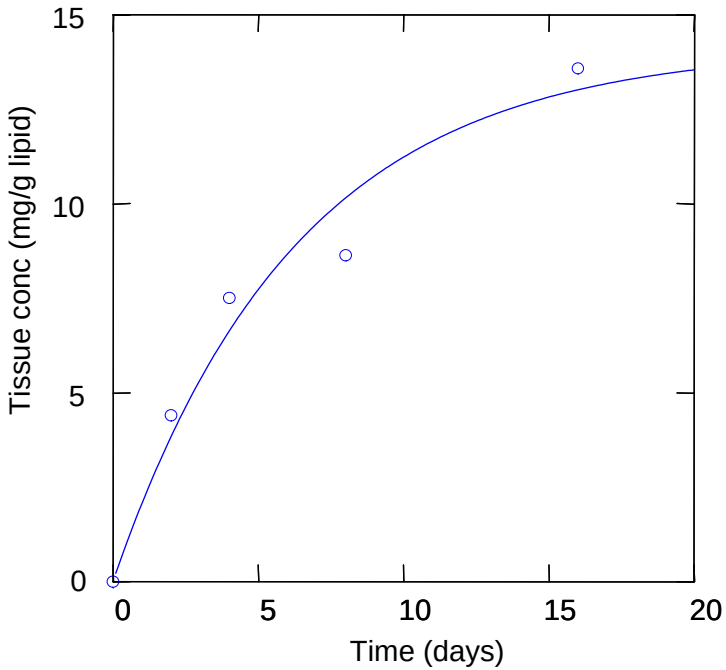
# *Uptake and elimination of fractions*

- Soxhlet extraction with methylene chloride
- Separate into saturates/monoaromatics and PAH/PASHs via activated alumina column chromatography
- Analyze by GC-FID and GC-MS

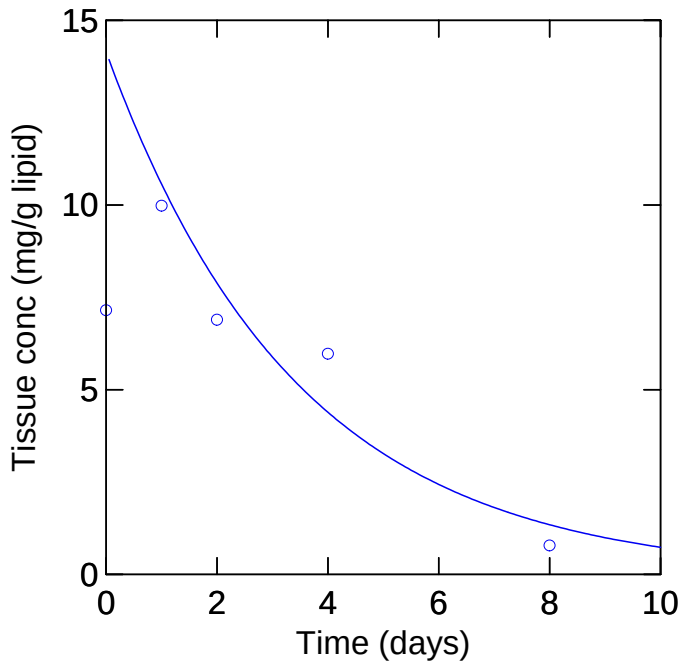
# *Uptake and elimination of fractions*

1) Uptake and elimination generally follows a one-compartment first-order kinetic model

Uptake of F3a PAH/PASH by E. andrei




Elimination of F3a PAH/PASH by E. andrei



# *Uptake and elimination of fractions*

2a) Both saturates and PAH/PASHs taken up by earthworms

- Increase in saturates in boiling point range corresponding to fraction tested
- Compounds in the PAH/PASH fraction were mainly alkyl-PAHs
- Once a maximum tissue concentration reached, the concentration decreases (metabolism?)

# *Uptake and elimination of fractions*

2b) For all fractions, the ratio saturates:PAH/PASHs is ~80:20

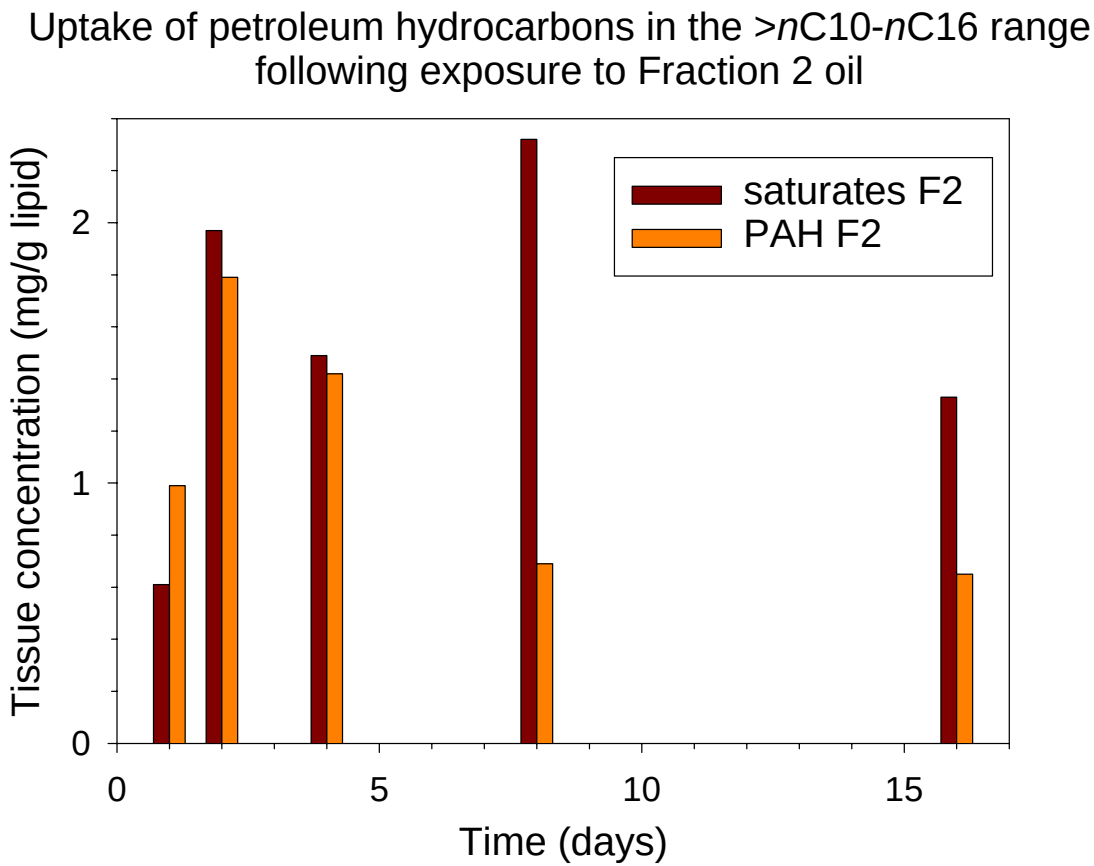
- PAH/PASHs disproportionately taken up by earthworms

  (tissue concentration PAH/PASHs ≥ saturates)

# *Uptake and elimination of fractions*

3) Uptake kinetics:

- Uptake of F2 and F3a saturates slower than PAH/PASHs; uptake rate approximately the same for F3b
- Lighter fractions taken up more quickly than heavier fractions



# *Uptake and elimination of fractions*

| | Approx. Time to reach maximum concentration | |
|---|---|---|
| | Saturates/monoaromatics | PAH/PASHs |
| **F2** | 8-16 days | 2 days |
| **F3a** | >64 days | 16-32 days |
| **F3b** | 64 days | 64 days |

# *Uptake and elimination of fractions*

3) Uptake kinetics

- Differences in the time to reach maximum body burden might explain the less-than-additive interaction of binary combinations of fractions
  - I.e., with F2F3a combinations, F2 will be taken up within 8-16 days, after which the concentration decreases; F3a concentrations do not reach a maximum until 16-64 days

# *Uptake and elimination of fractions*

4) Elimination of F2 and F3a occurred within approximately 8 days;  F3b >8 days

# *Uptake and elimination of fractions*

F3aF3b, F2F3a

- Tests on-going.  Data will be available by May-June 2005.

- Results of binary tests will indicate if there is a change in uptake due to the presence of a second fraction

# *Repeat toxicity tests*

- Repeating acute toxicity tests with *E. andrei*: F2, F3, F3a, F3b, F2F3a, and F3aF3b; and reproduction tests with F3, F3a and F3b to confirm toxicity
- Chemical analysis: same method used with uptake tests ($MeCl_2$ extraction, separation into saturates and PAH/PASHs, GC-FID)
- Tests on-going

# *Preliminary Conclusions*

- Binary tests
  - Fractions less toxic to earthworms if a second fraction present
  - Uptake test with binary combinations of fractions will indicate possible cause(s) for this change in toxicity

# *Preliminary Conclusions*

- Uptake Tests
  - Test indicates that PAHs contribute disproportionately to the body burden of the oil fractions; suggests that PAHs responsible for toxicity. Mainly alkyl-PAHs present.
  - Uptake kinetics might explain less-than-additive toxicity of binary combinations.

# *Preliminary Conclusions*

- Uptake tests
  - Uptake kinetics have implications for the interpretation of toxicity test results; to achieve maximum (and effective?) body burdens a longer test duration is required for the heavier fractions
  - Standard acute method has 14-d duration; our studies used 28-d duration; uptake tests suggest a duration up to or greater than 64 days for F3a and F3b. Our studies confirm the need for >28-d test duration for F3a and F3b.

# *Preliminary Conclusions*

- Uptake tests
  - Same applies for reproduction tests—adults exposed for only 28 days.  F3a and F3b exposed earthworms will not reach maximum body burdens within that time.
  - Pre-exposure studies recommended

# *On-going work*

- Acute and reproduction test repetitions
  - Increase confidence in toxicity values
  - Correlate toxicity to total, saturate and aromatic PHCs in soil

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