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Qualitative LC-MS/MS method for the detection of *desmethylbromethalin* in adipose tissue

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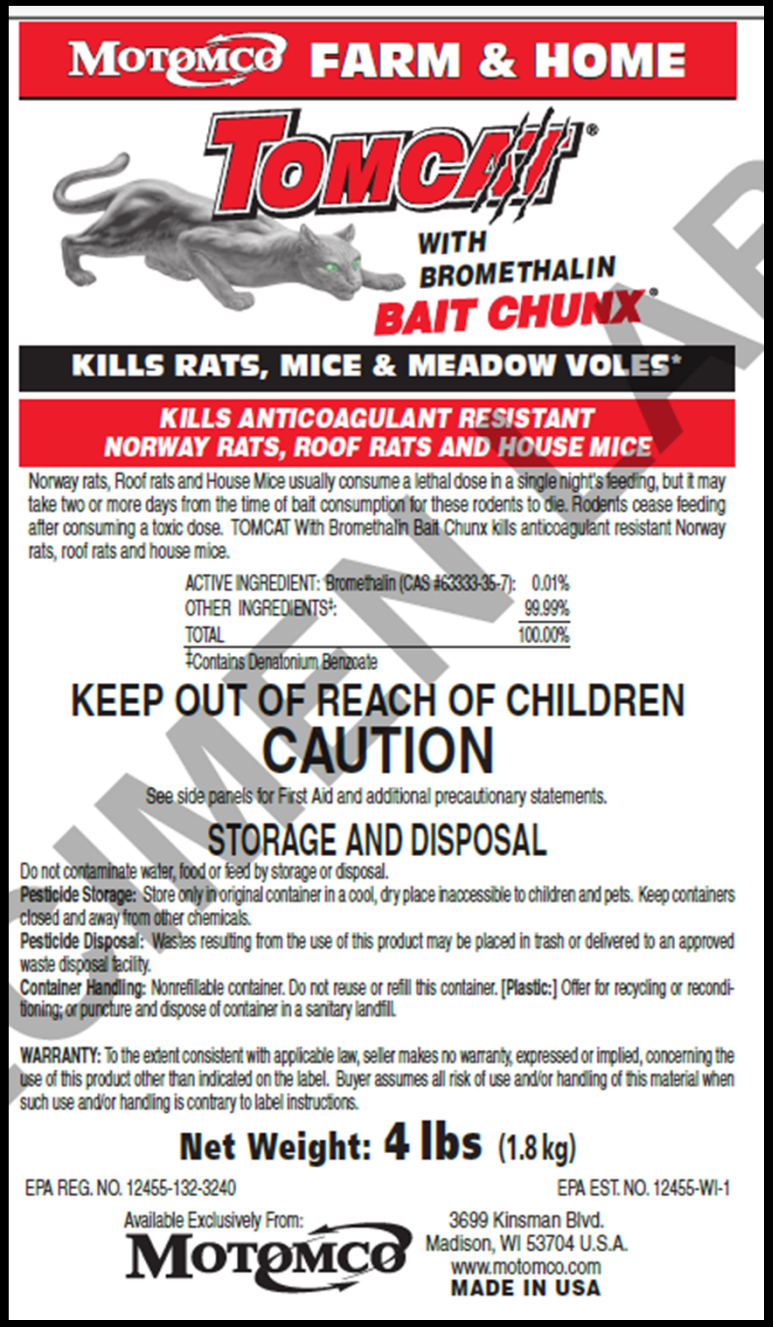
Rodenticides

- Long-acting anticoagulants
- Cholecalciferol
- **Bromethalin**
- Phosphide rodenticides

Assault
Fastrac
Gladiator
Rampage
Tomcat
Trounce
Vengeance



MOTOMCO FARM & HOME

TOMCAT®

WITH BROMETHALIN

BAIT CHUNX®

KILLS RATS, MICE & MEADOW VOLES*

KILLS ANTICOAGULANT RESISTANT NORWAY RATS, ROOF RATS AND HOUSE MICE

Norway rats, Roof rats and House Mice usually consume a lethal dose in a single night's feeding, but it may take two or more days from the time of bait consumption for these rodents to die. Rodents cease feeding after consuming a toxic dose. TOMCAT With Bromethalin Bait Chunx kills anticoagulant resistant Norway rats, roof rats and house mice.

ACTIVE INGREDIENT: Bromethalin (CAS #63333-35-7):	0.01%
OTHER INGREDIENTS‡:	99.99%
TOTAL	100.00%

‡Contains Denatonium Benzoate

KEEP OUT OF REACH OF CHILDREN

CAUTION

See side panels for First Aid and additional precautionary statements.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Store only in original container in a cool, dry place inaccessible to children and pets. Keep containers closed and away from other chemicals.

Pesticide Disposal: Wastes resulting from the use of this product may be placed in trash or delivered to an approved waste disposal facility.

Container Handling: Nonrefillable container. Do not reuse or refill this container. **[Plastic:]** Offer for recycling or reconditioning; or puncture and dispose of container in a sanitary landfill.

WARRANTY: To the extent consistent with applicable law, seller makes no warranty, expressed or implied, concerning the use of this product other than indicated on the label. Buyer assumes all risk of use and/or handling of this material when such use and/or handling is contrary to label instructions.

Net Weight: 4 lbs (1.8 kg)

EPA REG. NO. 12455-132-3240

EPA EST. NO. 12455-WI-1

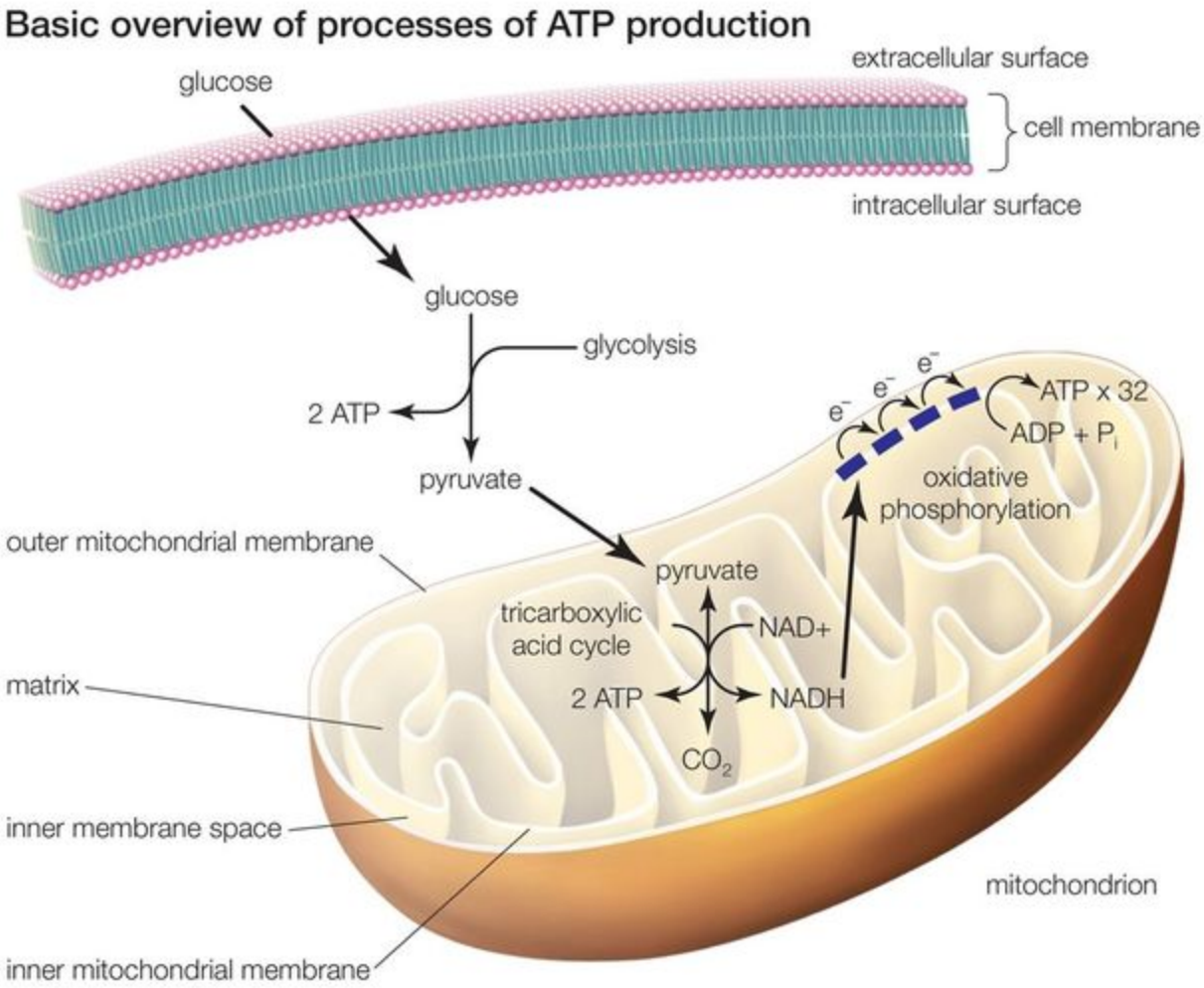
Available Exclusively From:

MOTOMCO

3699 Kinsman Blvd.
Madison, WI 53704 U.S.A.
www.motomco.com
MADE IN USA

Pharmacokinetics

- Rapidly absorbed in GI tract
- Peak plasma concentration 4-6 h
- Rapidly metabolized in the liver
- Excreted in bile
- Entero-hepatic recirculation (25%)
- Minimal renal excretion



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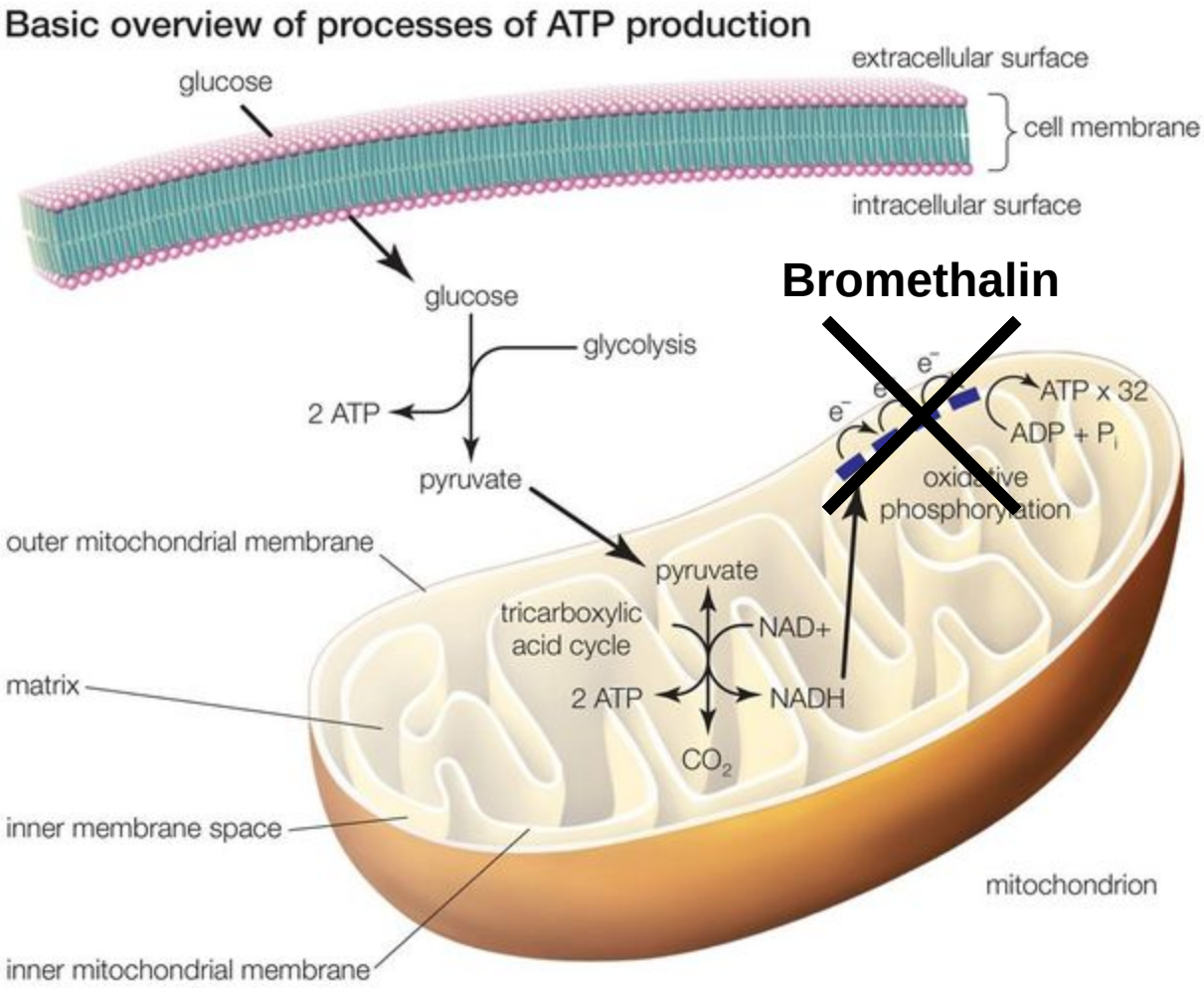

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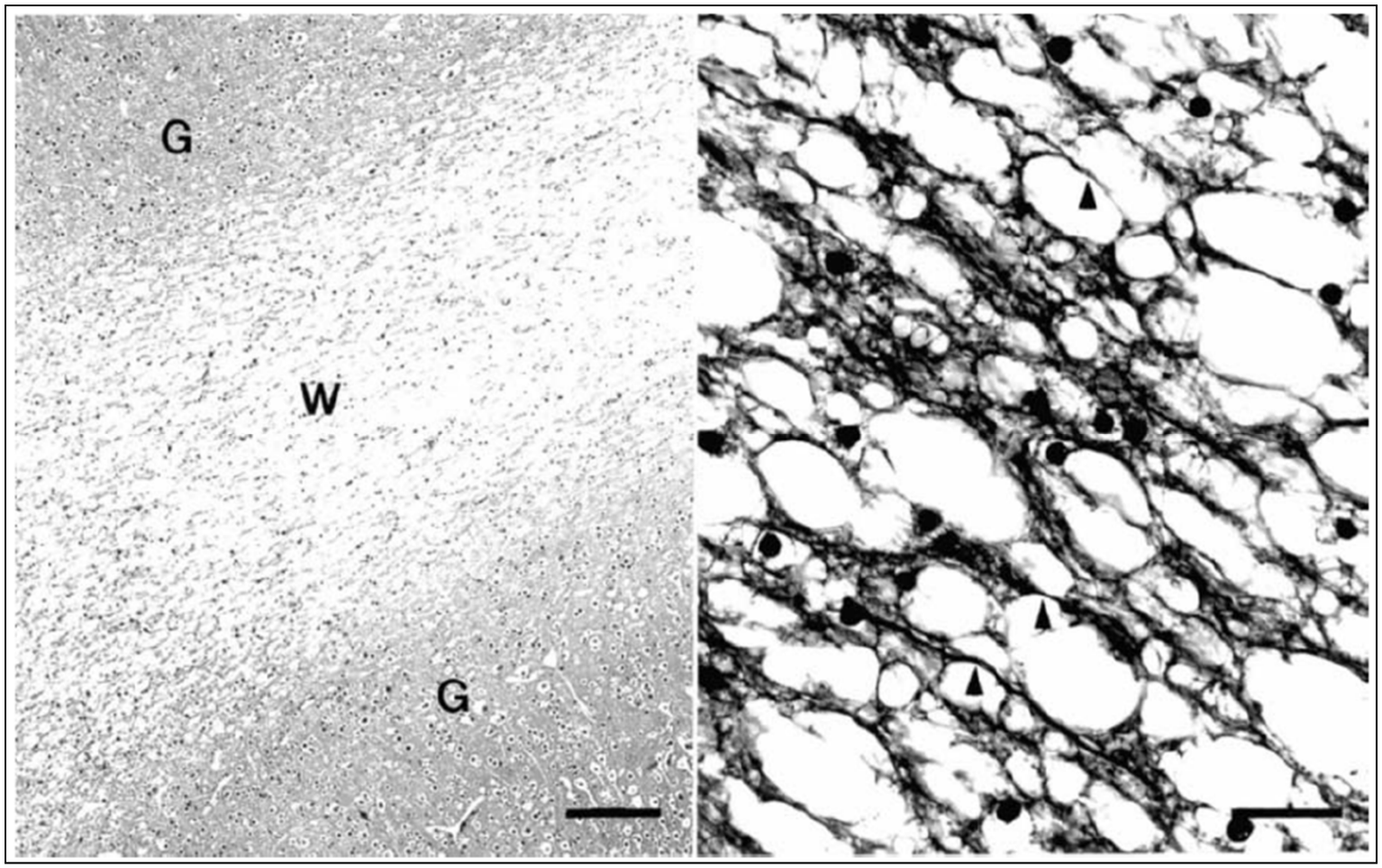
Mechanism of action

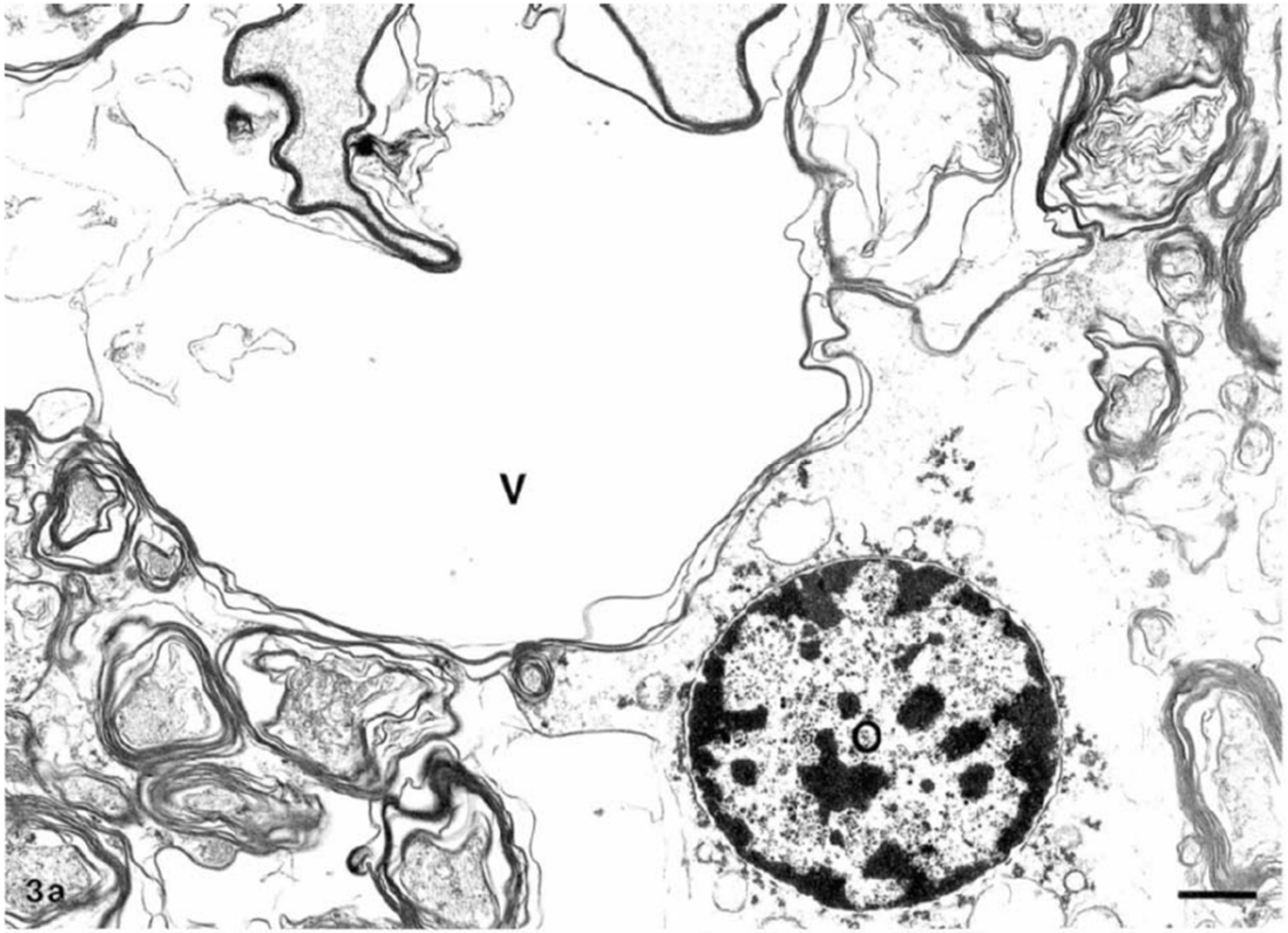
- Uncouples oxidative phosphorylation
- ⬇ATP production = ⬇Na^+/K^+ pump =
  ⬆intracellular Na^+ = edema
- Highly lipophilic and crosses BBB

Neuropathologic findings



Vet. Pathol 29:139-144 (1992)

Neuropathologic findings (EM)



Vet. Pathol 29:139-144 (1992)

Clinical signs (dose dependent)

High (10 hours)	Moderate (2-4 days)	Low (1-2 weeks)
Hyperexcitability Hyperesthesia Muscle tremors	Hyperexcitability Hyperesthesia Muscle tremors	Depression, vomiting, anorexia
Hyperthermia	Hindlimb paralysis Forelimb rigidity Paddling	Hindlimb ataxia
Seizures	Seizures	Paralysis, lateral recumbency
Death due to respiratory paralysis	Death	Coma, death

Toxicity

Dogs

- LD_{50}: 2.38-3.65 mg/kg
- MLD: 2.5 mg/kg
- Clinical signs reported at 1 mg/kg

Cats

- LD_{50}: 0.54 mg/kg
- MLD: 0.45 mg/kg
- Clinical signs noted at 0.24 mg/kg

Diagnosis

- History of exposure
- Clinical signs
- Toxicology testing
- Post-mortem lesions

Serum biochemistry and CSF: NSA

Bromethalin testing at the California Animal Health & Food Safety Laboratory System, UC Davis School of Veterinary Medicine, Davis, CA

Year	2013	2014	2015	2016
Total number samples	43	52	95	157
Number of positive (or trace) samples	5	8	50	52
% positive	11	16	53	33

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Methodology

- Method developed by California Animal Health & Food Safety Laboratory System, UC Davis School of Veterinary Medicine, Davis, CA (2013)
- With Mike Filigenzi technical guidance we installed the method in our lab at AHL.

Methodology

- Procedures requires 0.5 g of tissue.
- Tissue is homogenized with ethyl acetate using a Geno/Grinder®
- Homogenate is centrifuged, supernatant is poured off and dried down under nitrogen.

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Methodology

- Lipids are removed using an (QuEChERS) Agilent Enhanced Matrix Removal (EMR)-Lipid sorbent extraction technique
- Technique is two step process with the first removing of the lipids using dispersive SPE and the second (polish step) uses MgSO4, separates water from the acetonitrile.

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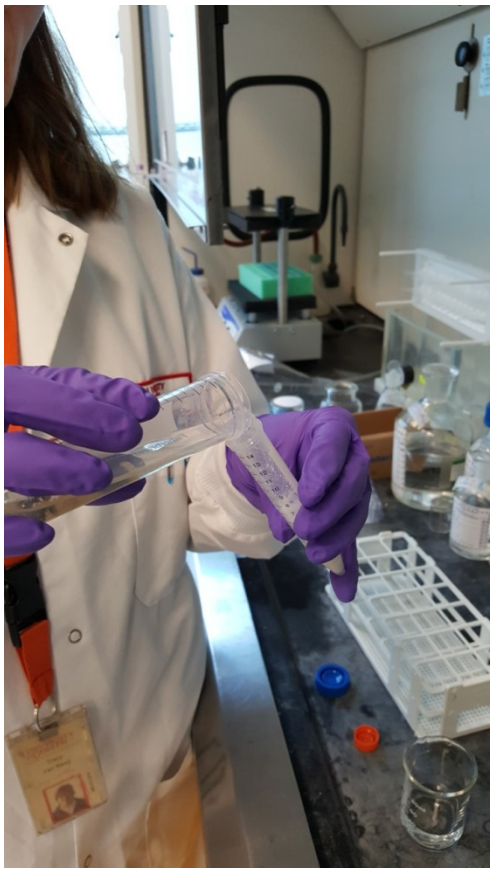
Methodology

- The extract is kept warm in a water bath.
- Acetonitrile is added to the extract, mixed and then added to the EMR-lipid tube and gently mix well.
- Solution is centrifuged, supernatant poured off into the polish tube which is mixed and centrifuge



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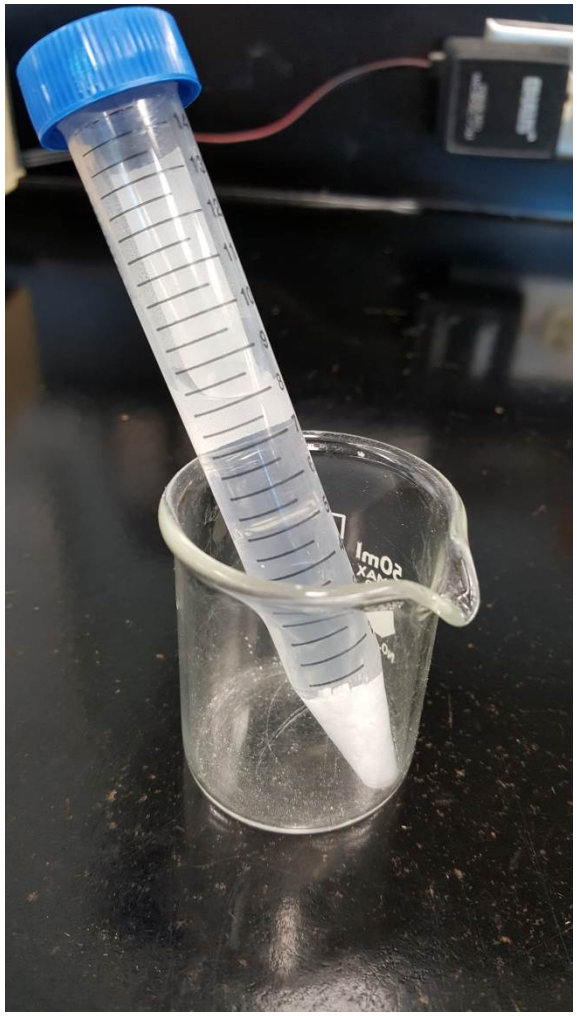
Methodology

- The upper acetonitrile layer is transferred to a culture tube and dried under nitrogen.
- Reconstitute with methanol, filtered and transferred to a autosampler vial for LC-MS/MS analysis.



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Instrumentation

- Instrument: AB Sciex API 4000 LC-MS/MS system , Agilent 1100 LC, Shimadzu SIL-20 AC autosampler.
- Column: InfinityLab Poroshell 120 EC-C18 column, 3.0 x 100 mm, 2.7µm fitted with InfinityLab Poroshell 120 EC-C18 guard column, 3.0 x 5 mm, 2.7 µm
- Mobile phase: A: 0.1% formic acid in nanopure water,
  B: 0.1% formic acid in acetonitrile
- Gradient Elution: 20-95 % B, 20 min.
- Flow rate: 700 µL/min
- Column temperature: 50 ^{o}C
- Injection volume: 10 µL

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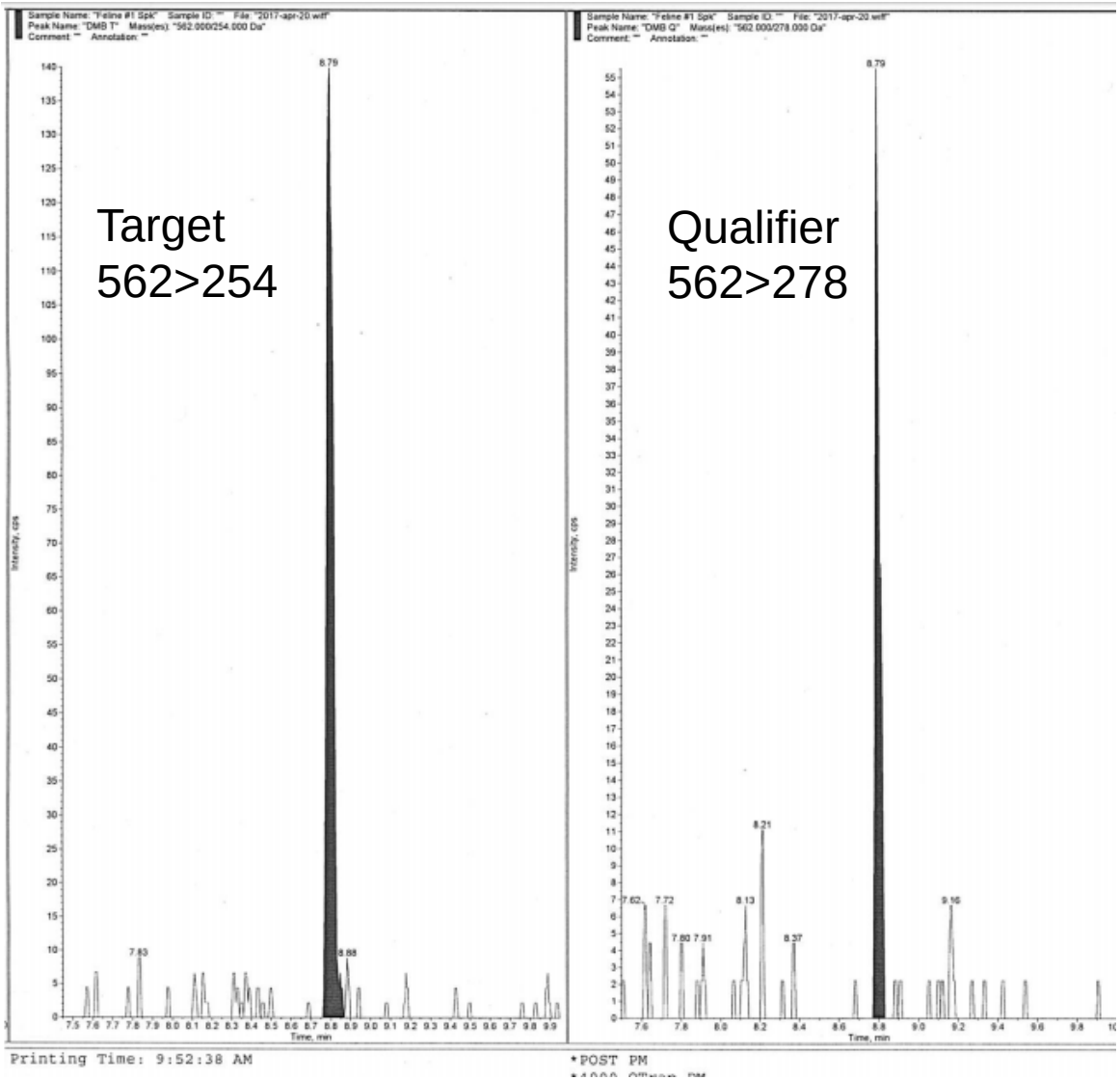
Spectrum



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Validation

- Qualitative result (positive/negative) based on instrumental detection
- Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods (AOAC)
- Determination of cut-off level
- 10 replicates at 7 fortification from 0-2.5 ppb (70 determination)
- Positive result – peaks (T and Q) S/N > 3, pass the EU criteria (Section 17.1, 2002/657/EC) where the designated ion transitions are present at the correct ratios relative to the spiked control.
- Plot % positive response vs fortification concentration

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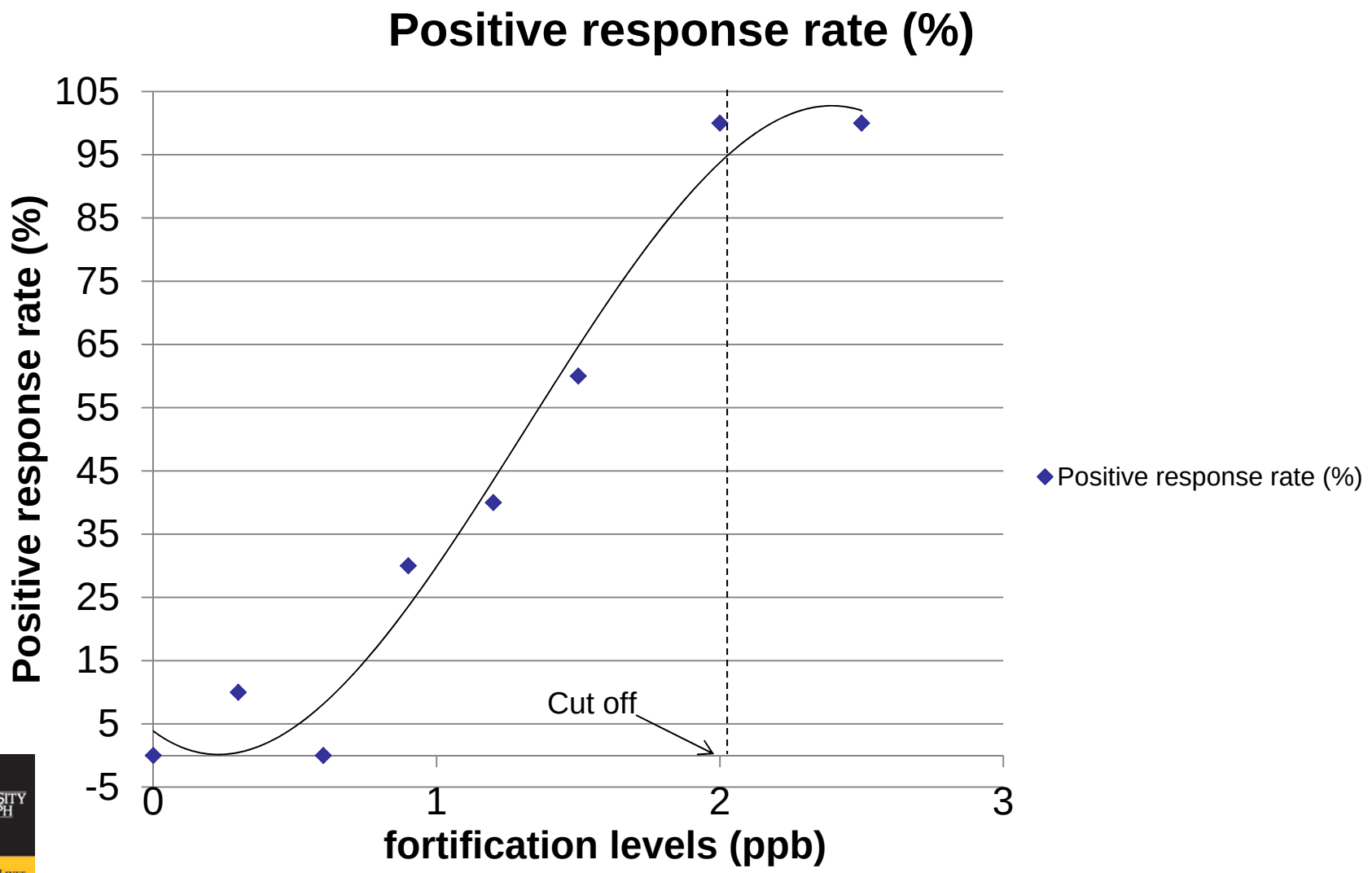
Validation



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Validation summary

- Cut-off level determined by positive response rate plot: 2 ppb
- Limit of detection (based on average S/N > 3): 0.9 ppb
- Sensitivity rate (from plot @ 2 ppb): 95%

Conclusion

- Analysis for desmethylbromethalin by LC-MS/MS is a sensitive, specific, cost effective and efficient method for establishing bromethalin exposure.
- EMZ lipid sorbent extraction technique is effective for the low level extraction of DMB from fat.

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Acknowledgements

- Mike Filigenzi, CAHFS, UC Davis
- Tracy Van Raaij, AHL
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