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

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

Contains Difenacoum (80F)

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

Container Handling: Non-refillable container. Do not reuse or re-fill 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

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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
Mechanism of action

• Uncouples oxidative phosphorylation

• \( \downarrow \) ATP production = \( \downarrow \) Na\(^+\)/K\(^+\) pump = \( \uparrow \) intracellular Na\(^+\) = edema

• Highly lipophilic and crosses BBB
Neuropathologic findings

Neuropathologic findings (EM)
# Clinical signs (dose dependent)

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

<table>
<thead>
<tr>
<th>Year</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number samples</td>
<td>43</td>
<td>52</td>
<td>95</td>
<td>157</td>
</tr>
<tr>
<td>Number of positive (or trace) samples</td>
<td>5</td>
<td>8</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>% positive</td>
<td>11</td>
<td>16</td>
<td>53</td>
<td>33</td>
</tr>
</tbody>
</table>
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.
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.
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
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.
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 ºC
- Injection volume: 10 µL
Spectrum

Target
562>254

Qualifier
562>278
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
Validation

Positive response rate (%) vs. fortification levels (ppb)

- Cut off
- Positive response rate (%)

Graph showing the relationship between positive response rate (%) and fortification levels (ppb), with a cut-off point indicated.
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 desmethylobromethalin 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.
Acknowledgements

• Mike Filigenzi, CAHFS, UC Davis
  • Tracy Van Raaij, AHL
  • Liz Karn, AHL
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