DETERMINATION OF ACRYLAMIDE USING IMMUNO-ENZYMATIC METHOD IN COMMERCIAL DOG AND CAT FOOD

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Acrylamide is an essential industrial and scientific chemical. It is estimated worldwide production more than 200 million kg/year. The acrylamide monomer is a white, odourless, crystalline solid at room temperature. One of the latest discovered neurotoxic and carcinogenic substances in foodstuffs is acrylamide.
Acrylamide formation

- The major acrylamide formation mechanism in food involves the Maillard reaction of the amino acid asparagine with reducing sugars.
- Solubility of acrylamide in solvents is variable, highest in water.
Structure of acrylamide
Acrylamide (CH$_2$=CH-CO-NH$_2$; 2-propenamide)
Toxicological profile
(Absorption, distribution, metabolism, excretion)

- Rapid and complete absorption by oral, inhalation and dermal routes in a range of species
- Rapid distribution to a range of tissues and organs
- Accumulation in liver, kidney, testes, epididymis, skin and red blood cells
- Metabolised to Glycidamide
- 60% dose excreted in urine in 24 h
- Information on bioavailability from foodstuffs as consumed needed
- *Animals-Incomplete information available*
Acute Chronice dose studies

- Oral LD$_{50}$ in rats, mice, guinea pigs and rabbits 107-203 mg/kg bw
- Skin and eye irritant and skin sensitization
- Main effect non-lethal reversible neurotoxicity (peripheral nerves)
Clinical signs

- sweating,
- nausea,
- myalgia,
- speech disorders,
- numbness and weakened extremeties
- abnormal manner
- vomited
- Convulsions
- ataxic and displayed hypermetria.
- nephropathy
Aim (Importance of Acrylamide Determination)

- Acrylamide is a known carcinogen in laboratory animals,
- impairing fertility in male animals
- and causing nerve damage in animals
- the starch-rich foodstuffs may be a neurotoxin in dogs
- The aim of this study is to detect possible acrylamides levels in dry cat and dog food
Materials and methods

- Analitical method which is immunoenzymatic method.
- The test is a direct competitive ELISA based on the recognition of Acrylamide by specific antibodies
- used commercial ELISA kit
- 42 cat and dog food were randomly collected and analyzed
- In order to analyse food samples for Weigh 2.0 g of a representative sample
- Extraction/Clean Up with Methanol/deionized water
- Derivatization Procedure (Derivatization Reagent reconstitution solution)
Results

- 33% (14 samples) presence of acrylamide have been found
- highest acrylamide level is 155 ppb
- the lowest is 45 ppb
- average acrylamide level is 87.35 ppb
Table 1, Statistical Findings

<table>
<thead>
<tr>
<th>Pet foods</th>
<th>N</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Std. deviation values</th>
<th>Std error</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>110.00</td>
<td>45.00</td>
<td>155.00</td>
<td>87.3571</td>
<td>8.42589</td>
<td>31.52681</td>
<td>993.940</td>
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</tbody>
</table>
Discussion 1

- Immunoenzymatic detection appears to be an effective analytical technique for use in cat and dog food due to its high sensitivity, low cost, and easy handling.
- Simple and low-cost alternative to chromatographic methods and is suitable and sensitive for the rapid determination of acrylamide in petfood samples.
- Immunoenzymatic method can be used as a standard technique on cat and dog food but it should be supported by more sensitive methods.
- It was below the level that would affect the health of cats and dogs.
The possibility of acrylamide toxicity occurring in pet animals, especially immature animals,
and should not be ignored when neurological cases are seen.
Further studies are needed to quantify the risk acrylamide in foodstuffs poses to dogs and cats.
REFERENCES

- Jerry M. Rice, Mutation Research 580 (2005) 3–20
- Pulley M T 1999 Acrylamide neuropathy