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Current and potential doping agents identified in customs and police seizures in Australia

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Introduction
For several years, ASDTL has assisted with the identification of suspected biopharmaceuticals seized by Australian authorities. NMI now has a formal agreement with the Australian Customs Service (ACS) to analyse seized items for performance and image enhancing agents that may be prohibited imports under Australian law. Relevant items are routinely subjected to immunoassay screening for six items listed as prohibited imports: human growth hormone (hGH), insulin-like growth factor I (IGF-I), insulin, erythropoietin (EPO), luteinising hormone (LH) and human chorionic gonadotrophin (hCG). We have examined the results of ACS seizures, and some relevant police investigations, during 2007 and 2008.

Materials and Methods
Thirty-four ACS seizures were reviewed, together with four ad hoc submissions from police services. Little information was supplied with the seized materials. Most items were marked as suspect hGH, regardless of any labels present. Intact vials were opened, reconstituted with water, and serially diluted over a wide range, using PBS containing 0.1% BSA. Dilutions were subjected to immunoassay for hGH, IGF-I, insulin, EPO, LH and hCG, using an Immulite instrument and reagents (Siemens Medical Solutions Diagnostics). Results at three dilution levels within the dynamic range of the assay were sought to support identification of the contents. Some samples were investigated further using LCMS-based analyses.

Results and Discussion – initial examination
Individual seizures contained between one and three distinct items in varying numbers. These may have been subsamples from large seizures, but this information was not often provided.
Of 43 distinct items (Table 1), 17 (39.5%) were hGH, containing from 3 to 15 mg per vial. Of these, 8 were labelled, including Serono Saizen (1), KeFei Biotech (2), GeneScience Jintropin (3), Mary Zhang (2). Unlabelled hGH commonly appears in a small glass crimp-top vial, with a blue plastic flip cap embossed with the words "FLIP OFF".

Fake hGH, labelled but with no active content, was also seen. In addition to homeopathic hGH and IGF-I (3), fake Jintropin hGH (Figure 1) was identified. Jintropin is also said to be widely counterfeited. hCG, 14 items between 2000 and 5000 IU per vial. All were labelled: Organon Pregnyl (8), with Bharat HuCoG (5) and Livzon hCG (2).

LH – not yet encountered. IGF-I and analogues – not yet encountered. EPO is unusual, last found March 2006 (EPOTREX-NP, South Korea). Insulin - not yet encountered. It may be easier to obtain than the other hormones, via diversion of legitimate supplies within Australia. This pattern suggests the illicit market in Australia is dominated by image enhancement rather than performance enhancement.

<table>
<thead>
<tr>
<th>TYPE</th>
<th>2007</th>
<th>2008</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGH - labelled</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>hGH – unlabelled</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Fake* hGH or IGF-I</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>hCG</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>No ID on immunoassay</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>ID on further analysis</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1: Breakdown of samples by content. *Fake - a labelled preparation which contains no detectable active content. Includes homeopathic hGH and IGF-I preparations.

Results and Discussion – novel findings

Nine unlabelled or poorly labelled items were found to contain no immunoreactivity for the six hormones of primary interest. Three were further investigated using LCMS:

1) Blue-capped vials, crudely labelled MedBotox. These contained a high MW major component, consistent with Botulinum toxin A.
2) Blue-capped vials, stated by the importer to be Melanotan. These contained a major component with a MW of 1024, consistent with Melanotan II, one of a group of synthetic analogues of α-MSH being developed as photoprotective drugs for use in dermatology (e.g., see www.clinuvel.com (sighted February 2009)). These drugs are widely abused as tanning agents, despite warnings from the US FDA and UK MHRA (see http://www.fda.gov/consumer/updates/melanotan090507.html (sighted February 2009) and http://www.mhra.gov.uk/NewsCentre/Pressreleases/CON031009 ) (sighted February 2009).

Again, these drugs are image, rather than performance, enhancing.

3) A large number of blue-capped vials obtained together with some accompanying information suggesting they may contain “PEG-MGF”. Mechano Growth Factor (MGF) is described by Goldspink et al. (2008) as a splice variant of IGF-I, particularly responsive to muscle damage and mechanical stimuli, with potential in the treatment of some muscular disorders. An internet search readily identified at least one supplier of an apparently pegylated peptide, PEG-YQPPSTNKNTKSQRRKGSTFEERK-NH₂ at
The structure described by this supplier matches part of the E peptide of IGF-I, which is significant in the differential function proposed for MGF. Pegylation and addition of the C-terminal amino group are presumably intended to improve bioactivity by retarding nonspecific proteolysis in circulation. LCMS analysis of this material (Orbitrap LCMS) yielded a mass of 2867.7, which corresponds closely to the exact mass of 2867.48 predicted by ExPASy. The preparation therefore appears to be the naked peptide sequence, contrary to specification. More investigation will be undertaken to confirm this. The safety and efficacy of this preparation in humans is entirely unknown. Whether it is imported for image or performance enhancement, the unknown properties of this preparation and the low quality of manufacture represent a considerable threat to the health of users.

Reference