Background
The Internet has many web sites where supplements may be purchased and the number of available products is steadily growing. For example a simple search of the Amazon.com site for key words gave the results shown in Table 1 where a significant increase can be observed even over a relatively short period of time.

<table>
<thead>
<tr>
<th>Key word</th>
<th>Number of hits Sept 2010</th>
<th>Number of hits Jan 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>supplements</td>
<td>184,114</td>
<td>198,058</td>
</tr>
<tr>
<td>sports supplements</td>
<td>2,043</td>
<td>8,097</td>
</tr>
<tr>
<td>Bodybuilding supplements</td>
<td>570</td>
<td>4,125</td>
</tr>
<tr>
<td>Steroid</td>
<td>17</td>
<td>5,588</td>
</tr>
<tr>
<td>Testosterone</td>
<td>429</td>
<td>3,251</td>
</tr>
</tbody>
</table>

Table 1: Search of website www.amazon.com for various keywords.

Other sites such as bodybuilding.com no longer sell products with steroids in them apart from 7-keto and DHEA containing materials. This appears to be a result of the FDA actually removing these items from such stores and requiring the company to send out letters requesting items to be recalled. The Amazon.com site now contains many “hard core” products but they do not list any ingredients and their links also do not provide any useful information. This makes it impossible to determine if a product has a prohibited substance as an ingredient without purchasing.

While many supplements contain materials (e.g. vitamins, proteins, minerals) with possible useful properties, there exists a set of “hard core” products which will continue to cause serious problems [1]. The problems that may be encountered include:
• Issues with contamination which may be at low levels due to coproduction of acceptable supplement ingredients with compounds that are banned for sport. These can be at low levels but if a urine is collected within a short time after ingestion or chronic use then a positive doping violation may occur [2,3];

• Deliberate adulteration to fortify a supplement with a substance that will provide the advertised effect and this can be with a high dose of the compound [4];

• Designer drugs which may or may not be listed on the label. It is not unusual to find a different substance in the formulation to the one on the label. An interesting feature is the attempt to hide their presence often by the use of unusual chemical naming on the label which makes the substance difficult to recognise by authorities. These drugs are most often steroids which have modified structures to make them unrecognisable by routine testing protocols used for doping control. We have often found that the steroids have poor chemical purity and appear to be reaction mixtures showing little or a poor attempt to purify the final products [5].

• Drugs that have been published and which may or may not be undergoing further trials. Often the literature describes properties that may be advantageous for doping and so these compounds may be synthesised clandestinely and then sold over the internet. Most of these do not even have data from a reasonable animal trial to support efficacy or safety. Some may be drugs undergoing human trials [6]. Good examples of untested drugs are the cannabinomimetics which may even still be legal in some countries but are readily and openly available on the internet.

•

An example study: The One

Experimental

$^1$H NMR was performed at the UNSW Mark Wainright Analytical Centre, Nuclear Magnetic resonance Facility using a Bruker Instruments Avance III - 400 MHz instrument with autosampler. The solvent used was D$_6$-DMSO. GC/MS was performed using a Shimadzu GCMS-QP2010 Plus, Agilent Ultra 1 column, 17 m, 0.20 mm, 0.11 um film thickness, Initial temp. 185C then 3C/min to 235C then 10C/min to 265C then 30C/min to 310 and hold for 2 min, injection 2 uL in split mode (10:1), 15 psi head pressure 0.8 mL/min flow helium.
Results

The label on this product obtained from bodybuilding.com before these products were removed from their shelves contained an ingredient named as 17α-methyl-etioallocholan-17β-ol-3-hydroxyimine which on translation may be 17α-methylandrostane-17β-ol-3-one oxime (the oxime of mestanolone). The dichloromethane extract of The One gave useful GC/MS traces after derivatisation with MSTFA/TMSI/EtSH and showed two compounds as pairs of isomers. Compound I had both isomers with a parent ion at m/z 463 and Compound II had these at m/z 462. Compound I had intense ion at m/z 143 and 1H NMR showed 3 methyl groups. Compound II did not have m/z 143 and 1H NMR showed 2 methyl groups.

Compound I was prepared from mestanolone by treatment with hydroxylamine hydrochloride [7] and the two syn and anti-isomers matched the compounds in the supplement. Syn and anti isomers occurred in about equal amounts.

Compound II had one mass less than compound I and therefore had either no nitrogen or an even number of nitrogen atoms in the molecule. The mass matches the hydrazone derivative but lack of m/z 143 and only 2 methyl groups leaves this out (checked by synthesis). Further calculations based on the mass and possible formulae indicated that this was the bis-oxime of 5α-androstan-3,17-dione and confirmed by synthesis from the dione by matching the mass spectrum and 1H NMR. This impurity is most likely derived from the starting material for the synthesis of mestanolone which had been carried along during manufacture and then formed during the preparation of the oxime.

The excretion of The One was performed by a male volunteer taking 2 capsules (50 mg) and separately collecting all urine samples separately for 48 hrs. Analysis using the routine GC/MS steroid screen used in doping analysis in our laboratory only found a trace of one of the oxime isomers in first 2 hours and 17α-methyl-5α-androstane-3α,17β-diol (the mestanolone metabolite routinely screened for in doping control) but the signal strength seems to be much lower than expected from the dose. No other metabolites were detected by GCMS. Further investigation needs to be carried out as metabolism of the oxime to reduced compounds would give compounds that may not be extracted easily during this routine screening procedure.
Conclusion
The “supplement” market is still active despite attempts to close some sites in the USA – they just moved elsewhere and products are now also available on sites such as Amazon.com which do not seem to have a physical address and are only web based. Many hardcore products contain steroids that are banned or closely related to banned steroids. Many contain quite impure products which may not match the substance(s) listed on the label. Identification of these products is needed so they may be included in doping control analytical procedures early in their sale period if laboratories closely monitor websites. This would allow drugs to be obtained and testing methods developed before they are widely used. The continued education of athletes and all persons around them on supplement use is paramount. “If in doubt – don’t” is a good rule since the athlete is held completely responsible for what they ingest. The professionals around them must also take responsibility – failure to do so is an abuse of trust.

References
Acknowledgements

Investigations into metabolism and detection in urine of new designer steroids found in supplements are supported by the Australian Government through the Anti-Doping Research Program of the Department of Prime Minister and Cabinet. Daniel Eichner (USADA) for assistance in importing many of the supplements which could only be shipped to a US address.