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The role of banned substance residue analysis in the control of dietary supplement contamination

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The potential for contaminated dietary supplements to result in a failed doping test remains a concern for athletes, trainers, and sporting authorities despite improvements to regulatory guidelines. Previous surveys of readily available supplements confirm that many are contaminated with steroids and stimulants prohibited for use in elite sport. Suggested responses to this issue include the complete avoidance of all supplements. Many athletes, however, use nutritional supplements to achieve effective training and also to ensure that daily nutritional requirements are met (e.g. recommended levels of vitamins and minerals). This ensures that the use of supplements is and will remain the norm for a range of sports. As a result, an alternative approach of rigorous testing of materials destined for use by elite athletes has been introduced in several countries. While the testing of final product for banned substances may help mitigate the problem, it will not help to remove the underlying issue of contamination. In this article we describe an alternative approach that uses appropriate quality assurance procedures backed up by testing to remove sources of contamination. The decrease in the incidence of contamination amongst supplement companies adopting such a system is explained, and contrasted with the relatively high incidences of contamination found in products that are not part of a quality system. These findings are of key importance to both supplement manufacturers and those involved in advising athletes about supplement use. Copyright © 2010 John Wiley & Sons, Ltd.

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Introduction

The potential for consumption of contaminated dietary supplements by an athlete, which could result in a failed drug test, is now well established.^[1–6] A number of studies of the prevalence of contaminated supplements have been published, suggesting that between 3 and 25% of supplements can contain low quantities of steroids or stimulants that are not specified on the label.^[1,7–8,12] The relevance of this contamination to a drug-tested athlete is clear with several studies confirming the potential for ingestion of contaminated supplements to result in a positive doping test.^[1–3,5–6] In a recent administration study in which male and female subjects ingested just 5 g of a creatine supplement that had been deliberately 'contaminated' with 2.5 µg of a nandrolone precursor (19-norandrostenedione), analysis of urine samples revealed that 25% of the subjects would have failed a drug test for nandrolone under World Anti-Doping Agency (WADA) rules.^[13] This represents a contamination level in a product of just 0.00005%. A recent study also described the presence of 19-norandrostenedione as a contaminant in a pharmaceutical product (an oral contraceptive pill containing norethisterone or norethisterone acetate).^[14] The levels of contamination were confirmed as being up to 0.05% (but this level did not exceed the maximum allowed concentration of 0.1%). These low quantities of contamination in a product raise an important issue for supplement quality control testing and for users of supplements and other products.

The prevalence of contamination in dietary supplements intended for use in sport could be related to the fact that some manufacturers and/or raw material suppliers have been responsible for producing or supplying both innocuous dietary supplements and products containing steroids or stimulants.^[8–11]

It is well understood that trace contamination between bulk materials is difficult to eliminate within a manufacturing plant. It should also be realized that even the most careful manufacturer is reliant upon the supply of non-contaminated ingredients if the risk of contamination is to be eliminated.

Quality control testing of supplements has increased over the years.^[15] In a recent publication, the results of the first three years of a system targeted at providing elite athletes with confidence in specific products was described.^[16] One of the conclusions was that a standard manufacturing quality system in itself could not prevent the release of potentially contaminated materials. This represents a logical approach given that most suppliers do not, and cannot, have complete control of the *total* supply chain. However, due to the limited amount of testing that can be reasonably undertaken for dietary supplements and the potential for point contamination even within a batch, periodic or ad hoc analysis of supplements *alone* cannot ensure the purity of a supplement.

An alternative approach is the use of a quality system which combines general good manufacturing procedures with education and an effective banned substance testing regimen. This is beyond the scope of food regulatory guidelines such as GMP (Good Manufacturing Practice), FDA (Food and Drug Administration), BRC (British Retail Consortium), ISO22000, etc.; such guidelines alone are *not* sufficient to prevent athletes from inadvertent doping risks. The quality system should identify hazards that are

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relevant to sport; while many manufacturers do carry out quality control (QC) testing it is unlikely that these routine controls will be relevant to trace banned substance contamination.

This article describes the development and application of such a quality system which allows the manufacturer to identify potential sources of contamination from the supply chain and to eliminate them. The effectiveness of the approach will be demonstrated by examining the results of supplements from suppliers utilizing this quality system with those from suppliers that do not carry out regular, banned substance testing as part of their QC procedures.

Quality System Requirements and Application

The supplement quality system approach is analogous to a process control loop frequently encountered in engineering (Figure 1). The desired output is 'low-risk' supplements (i.e. supplements that have been subjected to additional quality testing for banned substances) for release to the customer under the control of the quality system.

An initial assessment of the integrity of the manufacturer's existing QC processes (with respect to prohibited substances) is undertaken via a paper audit during which questions are asked about the controls in place, as well as discussions with the manufacturer about their procedures. Site visits are also undertaken where necessary. Final product testing for trace amounts of substances prohibited in sport using an accredited high-sensitivity assay is also undertaken. Products that do not show evidence of contamination are released for sale. In contrast, any contamination finding triggers further audit and investigation of the key stages of the manufacturing cycle with appropriate testing of raw materials, equipment, etc.

The method used to carry out product and raw material testing must have limits of detection (LOD) in the low ng/g or part per billion (ppb) region, and this LOD must be achievable for all matrix types that are to be analyzed (i.e. for solids such as bars, tablets, capsules, as well as liquids). The requirement for such a high-sensitivity assay is due to: (1) the potential for ingestion of low concentrations of some steroids to trigger a positive finding in an athlete's urine;^[1-6,9] (2) the fact that some supplements are taken in relatively large amounts; and (3) that contamination of products is rarely distributed homogeneously throughout a manufacturing batch.^[17] As such, screening a portion (e.g. a sample taken from a single container, or combined samples taken from several containers) from a given batch of product using a highly sensitive test allows an assessment of the likelihood of contamination in the remainder of that batch.

It is also essential that the method used is validated and accredited to a recognized analytical standard (ISO 17025). A

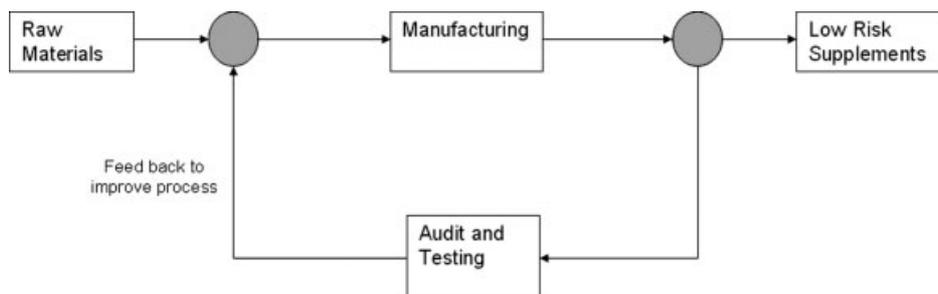


Figure 1. Process control loop for release of 'low-risk' products.



Figure 2. Management control diagram outlining the supplement quality system.

non-accredited method can give little reassurance as to the accuracy and validity of the test results obtained. In the quality system described in this article, an ISO 17025 accredited method with a limit of detection (LOD) of 10 ng/g for steroids and 100 ng/g for stimulants was employed. The testing protocol has been applied to the analysis of over 18 000 supplement samples over eight years.

The process control loop described in Figure 1 provides a mechanism by which improved management control and the routine production of 'low risk' supplement is achieved. This can be explained using the model shown in Figure 2.

In a situation where the manufacturer has inappropriate QC processes (e.g. inadequate cleaning procedures, use of unqualified raw material suppliers, lack of traceability for manufacture/distribution of products) and no final product testing (with respect to prohibited substances), there is a high risk of contaminated products being released for sale. This state can be referred to as 'unknowing incompetence'. In this situation neither the manufacturer nor the athlete is aware of potential contamination that may be in the product.

Progression towards 'knowing incompetence' involves the introduction of final product testing (for banned substances) and a review of the QC systems (via discussions with the manufacturer and site visit as needed). During this stage of the quality system approach, the integrity (with respect to prohibited substance contamination) of the manufacturing stages and supply chain is assessed and the most likely causative agents for contamination are identified for further testing. For example, some raw materials can naturally contain prohibited substances,^[18-19] and these will be monitored during this stage. If necessary, swab testing of equipment, hoppers, lines, etc., may also be undertaken to identify

and eliminate contamination from production equipment. At this point, the level of the problem can be understood and steps can be recommended to overcome these issues. Until potential contamination from the supply chain and/or manufacturing procedures is eliminated, the likelihood of a contaminated product being produced remains high and greater amounts of testing are required to monitor the situation.

Education also plays a vital role at this stage of the system. Contamination findings from the final product and/or ingredient analysis and, where applicable, equipment swabs, are shared with the manufacturer. Quality and production staff are informed how trace contaminants can enter the site and how this can be prevented. Actions are agreed to eliminate the causes and preventative measures are implemented: this may include modification of the manufacturing processes, the supply chain, equipment cleaning procedures, etc. In some circumstances, a switch to an alternative raw material supplier may be required or even reformulation to remove a problematic ingredient.

With increased maturity of the quality system the causative factors are gradually eliminated and the potential for contaminated product being released for sale diminishes. This stage is called 'knowing competence'. Continued high rates of testing are required including, in some cases, testing of raw materials to ensure as far as possible that the released batch is clear of contamination and that the product remains this way.

Once a high degree of confidence is achieved, the manufacturer enters the final phase of the management control process, which can be referred to as 'unknowing competence'. In this stage, a high degree of control can be retained, even with a reduced testing frequency (such as testing one in every three or one in every five batches, etc.).

This phased quality system approach in which sources of contamination are identified and eliminated in a stepwise and logical manner has the benefit of being cost effective as it minimizes the need to carry out analysis of materials that are unlikely to be contributing to the contamination problem. It also provides the manufacturer with a tool to educate staff and suppliers and improve the quality of ingredients, or if necessary remove them from the manufacturing process. Ultimately, the manufacturer can reduce its need for testing, and still ensure that its customers receive high-quality, low-risk products.

Trends with Supplements Made by Suppliers with an Appropriate Quality System

In 2008, 3579 supplement products and ingredients were analyzed by HFL Sport Science (HFL) as part of the manufacturer's quality system. These products comprised a broad range of supplements including, but not limited to: protein/whey products; energy/stimulant products; creatine; multivitamins and minerals; joint support formulations; weight loss aids; testosterone boosters; muscle gainers and muscle recovery aids; and training aids.

Of the final product samples tested (i.e. excluding any raw material samples), 0.4% showed contamination with steroids and/or stimulants. This value is significantly lower than the number of contaminated supplements reported in several surveys of products that did not undergo appropriate QC testing, where up to 22% of products were found to contain prohibited substances that were not declared on the label.^[1,11,17]

In 2009, 4567 supplement and ingredient samples were analyzed using the quality system approach (similar categories

of products as outlined above). Of the final product samples tested (i.e. excluding any raw material samples), 41 (0.9%) showed contamination with steroids and/or stimulants.

Of these 4567 samples, 4196 were received from manufacturers that had a well-established quality system approach in place. Only one sample from this category showed contamination (0.02%). In contrast, the remaining 371 samples were from manufacturers that were newly enrolled onto the quality system approach. Forty samples (10.8%) from this category showed contamination. It is anticipated that this number will decrease over time as the quality system approach matures (Case Study 1).

It should also be noted that analyses within the quality system were carried out *prior* to release for sale, thus further minimizing the likelihood of an athlete inadvertently consuming these products.

Selected Case Histories Highlighting Issues Encountered by Supplement Suppliers Using the Quality System Approach

Case study 1

A supplier of sports supplements (Supplier X) approached the HFL lab with a range of products to be tested. Analysis of final products showed contamination findings at a rate in excess of 3%.

Review of the manufacturing processes (provided by a third-party manufacturer) indicated inadequate QC procedures in terms of both the control of raw materials and also the management of cross-contamination within the manufacturing process. The third-party manufacturer had been using a range of QC testing technologies within its quality programme that had been drawn from the food industry and were primarily targeted at the verification of labelling. However, these measures were *inadequate* for the detection of contaminants relevant to sport. Supplier X took remedial actions, including changing the third-party manufacturer. Monitoring of these remedial actions has seen contamination findings fall to a negligible level (from 3% to less than 1%), and maintaining this even during extension of its product range and increase in production (Figure 3).

Case study 2

A capsule manufacturer Y requested banned substance testing on its finished products. Test results showed evidence for contamination with DHEA, which was not used at the facility. Initial investigations involved analysis of the bulk powder blend that had been used prior to encapsulation. These tests showed no evidence of DHEA contamination. Investigation was directed at the encapsulation machine, which had recently been purchased by the manufacturer. Analysis of swabs and washings from the equipment showed the presence of DHEA. Further investigative work revealed that the new piece of equipment was, in fact, a reconditioned machine that had been used several years previously to make DHEA products. Despite the passage of time, reconditioning, and cleaning, DHEA remained trapped within the equipment. Remedial actions included thorough equipment cleaning, and testing of additional washings and swabs to ensure that all traces of DHEA had been cleared. Ongoing monitoring of capsules made using this equipment continued to ensure that the capsules remained free from DHEA and other contaminants prohibited in sport.

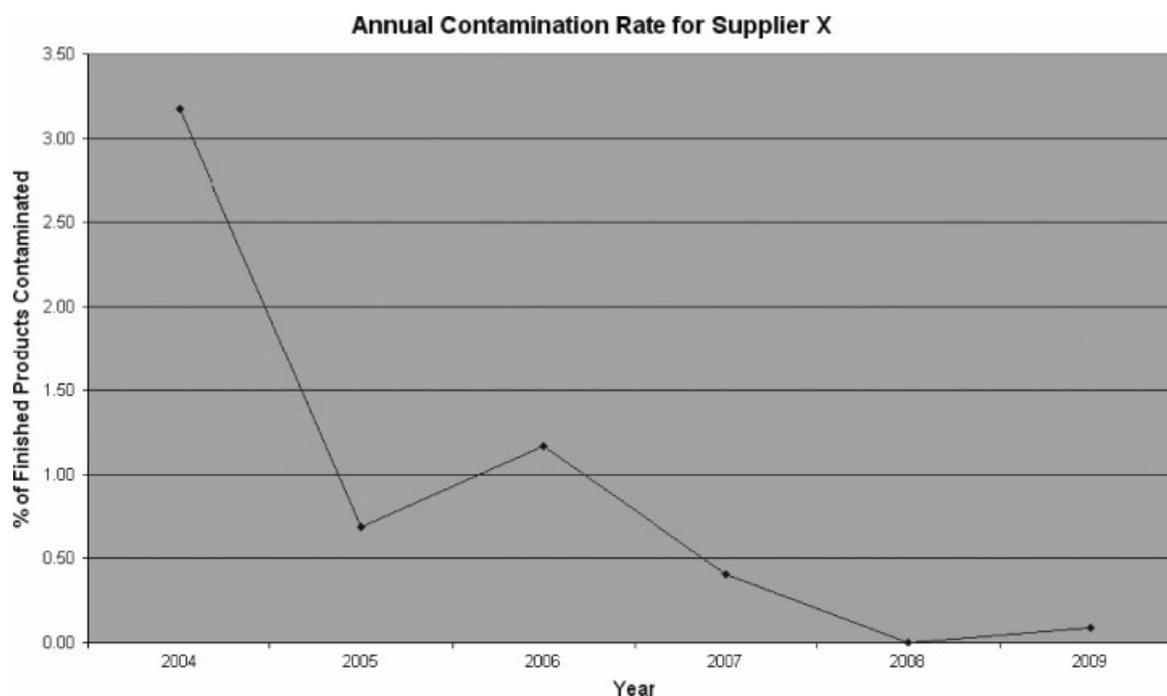


Figure 3. Annual contamination rate for Supplier X.

Conclusion

A QC system for supplement production cannot *entirely* remove the potential for contamination of nutritional supplements with low amounts of substances prohibited in sport. This reflects the difficulty manufacturers and suppliers have in controlling the entire production process and contamination issues are therefore likely to remain.

Where a good manufacturing system is supported by an appropriate and accredited prohibited substance testing regimen, however, significant improvements in contamination rates can be achieved. Manufacturers utilizing HFL's quality system approach have been shown to have contamination rates as low as 0.02%. Such manufacturers that adopt this approach typically show a significant decrease in contamination findings as the system matures.

Nevertheless, athletes remain entirely responsible for what they consume and should take appropriate measures when choosing supplements – selecting only those that have been made to high-quality standards and that have been appropriately tested. There is no 100% guarantee that tested products are *entirely* free of every prohibited substance but it is clear that regularly tested products made in facilities with an appropriate quality system offer a significantly reduced risk compared to non-tested products.

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