"Clenbuterol in meat. Development of a decision model for the discrimination between contaminated meat and pharmaceutical preparations. Part 2"

Dr. S. Sterk, Dr. K. Parr, Dr. M. Nielen (RIKILT Wageningen UR, the Netherlands)

Project overview:

Several elite athletes were tested positive for clenbuterol and claimed that these findings were caused by the consumption of meat containing clenbuterol. From several studies and EU monitoring programs it is shown that clenbuterol may be present in meat and after consumption of meat concentrations of clenbuterol in urine can be found. To discriminate between clenbuterol administrated via a pharmaceutical preparation or by ingestion of contaminated meat products research was started in 2012 (WADA 11A18SS). The focus of this study was to determine if there was a difference between the ratio of the two enantiomers (left- and right-- hand form of clenbuterol) in meat, and if after consumption of meat there was a difference between the ratio of the two enantiomers in urine when compared to the ratio in urine after illegal oral administration of preparations to humans.

In 11A18SS the proof is given that the hypothesis is feasible and the analytical methods are in place and capable to detect the differences. In the proposed project the focus will be on establishing a relation between the consumption of contaminated meat and a change in ratio of clenbuterol enantiomers in urine via a controlled experiment. The project will result in a decision model that can be used to assess the source of an adverse analytical finding for clenbuterol. A new technique focusing on untargeted analyses will also be tested on the acquired samples. This will take into account if there are any other changes in metabolic profiles after both ways of ingestion and will try to discriminate on this basis. This technique is known as metabolomics.

Results and Conclusions:

The aim of the project was to confirm the proof of principle from a former project with controlled human trials and to build a decision model to be used in sports doping analysis to distinguish AAF for contaminated meat from pharmaceutical preparations. Meat and liver were collected from an animal experiment. The meat and liver were used for a controlled human trial in which these tissues were consumed by volunteers. Other volunteers ingested a pharmaceutical preparation containing clenbuterol (racemic mixture) or enantio pure clenbuterol. Urine samples of the volunteers were collected for over a week after consumption. All urine samples were analysed using the

methods developed in 11A18SS project. The urine samples were also used for untargeted profiling experiments.

In meat from the animal experiment had S/R clenbuterol ratio of around 1 and 0.9 depending on the animal. This shows that the enrichment of R-clenbuterol in meat of treated animals is not a process that is stable, reproducible and comparable for individual animals and maybe also depends on the concentration administered to the animal. For bovine liver no information on the ratio was available before. Data from bovine liver showed that in liver the S-enantiomer is either enriched or R-clenbuterol very depleted. This is the opposite of the ratio in veal meat. This means that consumption of liver with incurred residues can possibly lead to an opposite ratio in human urine compared to the consumption of veal meat. It was also shown that preparation (cooking and baking) of the incurred meat and liver had little to no effect on the ratio determined prior to preparation.

In the administration studies a distinction based on the proportion of S-clenbuterol in the human urine samples was possible between those receiving liver (padmin=0.635 ± 0.004) from the other two groups, Spiropent® tablet (padmin=0.499 ± 0.001) respectively meat (padmin=0.509 ± 0.006). A distinction between the volunteers receiving meat and Spiropent® tablets cannot be made based on the enantiomeric composition, due to the reason that the ingested proportions of S-clenbuterol are too close to each other.

The analysis focusing on potential metabolites of clenbuterol in a targeted non-targeted design predicted a high theoretical number of metabolites/biomarkers. Unfortunately, not many of the predicted metabolites were found in the urine samples of the volunteers. Based on the outcome of the analysis and the statistical processing there is no grouping of the compounds possible at present. There is no underlying mechanism found what could be used to separate the different treatment groups. So it was concluded that it is not possible to use clenbuterol metabolites to discriminate between intentional and unintentional intake of clenbuterol using non-enantiomeric separation.

Overall it seemed that inter individual differences between elimination kinetics in bovine animals are present. This made setting an absolute threshold or guideline for discrimination not possible. The method developed in the project can be used when there are adverse analytical findings for clenbuterol to obtain additional information. When the S proportion clenbuterol in human urine resembles 0.5 (the S proportion in a pharmaceutical preparation) this is not definite proof of illegal use. However, if the S-proportion is higher than 0.59 this means that in 95% of the cases this is not due to administration of clenbuterol in (racemic) tablet forms.