Eggs are one of the basic ingredients in the human diet, so it is important to ensure the safety of this type of food. The appearance of the product is so important for the consumers that the food producers' dye the egg yolks using colouring agents in the form of feed additives registered for laying hens. Because not all of the dyes are available in the synthetic forms, they may be extracted from the plants containing them. In this way oleoresins, which are feed additives are produced. The direct addition of marigold flowers, peppers, alfalfa, carrots, tomato peel, corn meal, dried alfalfa and grasses, or crab shells? is more and more often practiced.

In the past, some food producers in China used banned toxic azo-dyes, attracted by their low price and availability. In 2006 in Hong Kong dye Sudan Red IV was detected in hens' and ducks' eggs, at a level reaching 300 µg/kg. It is not exactly known, how the dye was administrated to the animals. Probably it was intentionally added to feed intended for animals. This pigment belongs to the group of azo-dyes, banned as food additives but very often detected in chilli, red pepper, curry or turmeric. This is because the spices during the storage lose their colour and the addition of a stable yellow-orange or red azo-dyes give more intensive colours to the spices, which suggests a better quality and freshness of the product.

The dyes selected for the project can be enzymatically transformed in human body into carcinogenic aromatic amines. Sudan I is genotoxic; data on the toxicity of other azo-dyes are insufficient, it cannot be excluded that their effect is similar. The presence of toxic metabolites of industrial dyes in food may therefore pose a risk to the consumers. Because paprika, in which the banned dyes are found, may be further used to produce the oleoresins which are used as feed additives, unintentional administration of the banned dyes to animals is possible.

The aim of the project is to examine the degree of degradation of selected azo-dyes by selected intestinal hens bacteria and identification of metabolites formed. It is planned to carry out the tests using four kinds of bacteria, the most often isolated from laying hens (*Bacillus subtilis, Clostridium perfringens, Enterococcus* spp., *Escherichia coli*) and five toxic azo-dyes banned for use in food (Sudan I-IV, Para Red).

Our previous research was aimed to simulate the transfer of a genotoxic Sudan I from feed to eggs. The results were surprising. The transfer of Sudan I to eggs was significantly lower thanit was expected based on its physicochemical properties and the fate of the dye in the body of hens was not explained. Sudan I must have been transformed, because its concentration in the faeces of hens was significantly lower than in the diet. The results prompted us to continue the research because of the high toxicity of potential metabolites and /or degradation products of the dye.

The study will be performed using the electrochemical analyzer, capable of performing the oxidation and reduction reactions. It allows the simulation of metabolic processes taking place both in liver and bacterial cells. It enables to produce all possible metabolites, without conducting *in vitro* or *in vivo* tests. The results are easy to interpret, because there is no biological matrix interfering during the sample analysis. This technique is inexpensive and more and more frequently used in studies of the metabolism of xenobiotics. It allows reducing the research carried out with cell lines and animals, but cannot fully replace *in vitro* and *in vivo* tests.

After the simulation test of the metabolism of azo-dyes and development of method for its determination, the next step is designed to study the degradation process of the dyes by intestinal bacteria. The studies will be carried out with reference strains of bacteria *Bacillus subtilis, Clostridium perfringens, Enterococcus faecalis* and *Escherichia coli.* The results will be compared with those obtained for the field strains of the same types of intestinal bacteria, isolated from laying hens. The degradation of the dyes by the unspecified intestinal flora of laying hens will be also studied. Experiment will be made using the intestinal flora contained in the faeces of hens from different stocks in order to best reflect the real reduction of the dyes. The choice of laying hens as the source of the bacteria isolates that potentially degrade the dyes is justified by the scale of the consumption of poultry products and consumer preferences. The data on the azo-reduction of the dyes by reference strains and field isolates of non-specific intestinal flora of laying hens will allow the identification of potential threats to animal health and consumer health as well as identification and initial characterization of bacteria capable to degrade of these compounds.

So far, such studies utilizing the use of animals have not been conducted, nor the determination of all of the metabolites was possible, due to the difficulties in the appropriate sample preparation of the cell cultures media. Simulation of metabolic processes in an electrochemical analyzer is the answer to these problems. The method developed for the determination of metabolites will be used at a later stage in *in vivo* studies of their presence in eggs and animal tissues.