2.4. Risks related to the presence of antibiotic residues in food

Molecules that benefited from detailed research

For the last 60 years, antibiotics have one of the most used molecules in human therapy as well as in veterinarian therapy. For many years, synthesis on the subject have been published and the question of antibiotic residues in milk is considered a priority in this field (Ref. 46, Ref. 13, Ref. 30). The scientific community has a very large knowledge based on the scientific essays carried out for molecule testing and on the observation of the consequences of the use of these molecules at a wide scale.

The regulation evolution (see chapter on MRL) progressively required pharmaceutical companies to perform an increasing number of laboratory and "field" essays to ensure a good control of the risks identified when using a drug in human, as in veterinary medicine.

Direct toxicity

As regards to antibiotics, the most frequently named case of toxicity possibly linked to the presence of antibiotic residues in food is chloramphenicol. This molecule, after the discovery of aplastic anemia related to its use in human medicine, has been banned in Europe in veterinary medicine (see details below).

The other best known cases of toxicity due to the presence of veterinary medicine residues in food do not concern antibiotics but other medicines, such as clenbuterol (one of the rare toxicity cases related to the ingestion of food containing residues).

Toxicity of chloramphenicol: a textbook case

Today, chloramphenicol is the most studied antibiotic by the surveillance plans. Although its inherent toxicity has been relatively dramatized for different reasons, this antibiotic has indeed immunotoxic and low genotoxic possibilities. Its residues therefore go against all precautions.

Immunotoxicity, though very rare, is often fatal for human beings (aplastic anemia) and no dose-effect relation has been proven (which does not mean none exist). It has recently been underlined that the therapeutic use of chloramphenicol by humans as a collyrium (equivalent to10 mg per dose and at the origin of the first known cases) creates less than one untoward reaction a year throughout the world. Systemic absorbtion from the eye is very low. If estimated under 1% of the dose, the quantity absorbed by the organism could be evaluated at approximately a maximum of 100 micrograms, equivalent to one dose above the residual doses ingested with contaminated food.

Regarding the possible genotoxicity of chloramphenicol, *in vivo* experiences on rodents showed low genotoxic effects. However, the concentration level was approximately 25 times more than therapeutic doses (the latter being very much higher than residual doses).

The RIVM (Netherlands institute for public health and environment) calculated that shrimps contaminated by chloramphenicol between 1 an 10µg/kg (dose found under the surveillance plan) would in the worst case have a theoretical effect 5000 times less than the maximum tolerated risk (increased cancer risk of one for one million in the population). These results are representative of many toxicologists opinion: chloramphenicol, in the form of traces, is probably innocuous and to date has had no part in any adverse effects.

Then, why is the chloramphenicol monitored so much? Simple precaution? Of course, but not only. This molecule – certainly suspect – also has against it to be in the public domain: it is in no industry's interest to sponsor the research which could experimentally determine its toxicity limits and therefore define a MRL to authorize its use. Note that possibly veterinary medicines are not the only source of chloramphenicol in food.

First, chloramphenicol is a natural compound produced by the micro-organism *Streptomyces venezuelae*. This mould is present in many soils and chloramphenicol has been isolated. Secondly, in certain countries, such as China where chloramphenicol was only forbidden recently (2002) in veterinary medicine, but still used in human medicine, contamination of the environement is inevitable. Environment contamination by chloramphenicol from wastes of diverse origin has already occurred: thus, in Germany, in 1999, chloramphenicol was detected in certain surface waters and sewage treatment effluents. These results explain why the notion of "zero tolerance" is quite difficult to apply to such an ubiquitous molecule and in particular with such detection methods as performant as nowadays.

Carcinogenic and mutagen effects

As regards to carcinogenic and mutagen effects, antibiotics are generally not among the hazardous molecules (unlike certain antiparasitics).

Definitions

Carcinogenic effect: *from the Greek carcinos, describes a physical, chemical or biological event able to cause the development of cancerous tumors on humans or animals.*
Mutagen effect: *process of changes (code modifications) in the genetic sequence. A repeated mutagen effect on eukaryote organisms is eventually related to a carcinogenic effect risk.*
Genotoxicity: *any form of toxicity directed against the cell's hereditary material A major consequence of genotoxicity is the mutagen effect.*
Immunotoxicity: *toxicity for the immune system (production of antibodies and immunity cells) resulting in hypersensitivity (allergy, anaphylaxis) or, less often, immunodepression.*

Let us however note the case of nitrofurans used at high and lengthened doses, which carcinogenic and mutagen effects were proven on animals and during genotoxicity tests. These molecules are forbidden in most countries for veterinary use in animal production.

The other substances considered as potentially carcinogenic are: quinoxaline, carbadox and olaquindox, antibiotic additives which are now forbidden.

Among the antibiotic molecules still authorized in most countries, there is no substance with a known direct carcinogenic effect.

Teratogenic effects

The possibility of the presence of residues in food with an effect on the embryo carried by a woman (newborn malformations) is a major question.

Definition

Teratogenicity: *in utero embryo toxicity resulting specifically in the development of malformations. An embryo is sensitive to teratogenicity only during a limited period of the gestation (at the beginning). Teratogenicity is only among one of the aspects of toxicity for the embryo (embryotoxicity).*

Teratogenicity can be inducted by one single exposure to the molecule. It is an acute form of toxicity.

Concerning medicine, teratogenic effects have been proven for certain sulfonamides, many organic phosphorus and carbamates, antiparasitics, dexamethasone and antifungal such as griseofulvin. For sulfonamides, it must be underlined that the doses possibly at the origin of teratogenic effects are thereapeutic doses used for human beings which are on an altogether different scale to residual doses that can be found in food.

Allergies

The question of the potential effect of the presence of antibiotic residues in food has been under research for many years and is posed at two levels:

- Sensitizing effect that would be at the origin of allergy. It is the case, for example, of a first antibiotic treatment made on an individual.
- Start effect that would correspond to an allergic reaction on an individual already sensitized.

Cases of allergies on people treated with antibiotics are not rare. Antibiotics and certain anti-inflammatory drugs are probably the molecule families that have been the most under observation and research for their allergic effect on human beings. Among the antibiotics the most often mentioned for having caused allergies in human medicine are: amoxicillin, trimethoprime-sulfamethoxazole, ampicilline, cephalosporins, erythromycine, penicillin G, as well as strepthomycin, to a lesser extent neomycin, nitrofurans, spiramycin, novobiocin and tetracylines.

Many of these antibiotics at the origin of allergies related to treatments on humans are also used on farms. Thus there is a real potential risk of allergy in case of ingestion of food with residues. Actually, this risk mainly exists with penicillins which are among the most immunogenic and the most often used.

There are few publications presenting cases of allergies due to the presence of residues in food, approximately twenty in ten years throughout the world. Most of them concern penicillin residues. All these adverse reactions, noticed after ingestion of food containing residues, are limited to skin rashes, except for the case of one person, exceptionally sensitive to penicillin and who had a non-deadly anaphylactic shock.

Relating an allergic reaction to the presence of residues in food being very difficult, the number of allergies possibly related to residues in food and declared as such seems very limited. Hypothesis or suspiscions are more often mentioned. That’s why tests were performed on human volunteers known as allergic to penicillin, in order to study the consequences of consuming food with known contents in residues.

Milk in which penicillin G was added at 75 micrograms/l (more than 18 times the MRL) was administered to thirteen already allergic volunteers: only four of them had positive reactions (urticaria), three were uncertain and six had no reaction (Ref. 54). In another test, nine allergic volunteers ate pork treated with penicillin G and containing 6 micrograms. Two of them feeled itching sensations (Ref. 41).

It is much more frequent to see allergies due to natural constituents of food rather than to the presence of residues.

Effect on bacterial flora

Bacterial flora present in the human digestive tract plays a considerable role in human health. Its role was highlighted by the researches on the effect of lactic ferments.

The effect of the presence of antibiotics, further to a human medical treatment, has clearly been proven and is a real preoccupation in human medicine. It is therefore important to consider the effects of the presence of residues in food on the normal flora of the human digestive tract. This is why the MRL setting studies include the effect of residual doses of antibiotics on different bacteria strains usually present in the human digestive tract (see MRL chapter). **Respect of the MRL also aims at guaranteeing the absence of measurable effects on the human bowel flora**.

Study of the effect of an antibiotic on the barrier flora

A study on a chemostat (reconstituted human flora) carried out in 2004 explored the effect of ciprofloxacin on the barrier flora, using as marker the colonization by a salmonella which was unable to colonize intact flora (Ref. 11). The results show a sensitivity of the barrier flora from 0.43 micrograms/ml, corresponding to a total ingestion of 0.5 mg of the antibiotic in one take.

The MRL of the closest veterinary molecule, enrofloxacin, is of 100 micrograms per litre of milk, that is to say 0.1 micrograms/ml: this is four times smaller than the active concentration described in the study. Even if somebody takes a lot of milk, this has no influence on the concentration which is the critical factor (an not the total ingested quantity).

Transmitted or acquired antibioresistances

The effect of antibiotics use on farms on the emergence of bacteria resistant to human medicine treatments is an essential question studied by the scientific community for several decades (Ref. 38, Ref. 26, Ref. 61, Ref. 62). This question has become even more important since the rate of resistant bacteria has increased whilst the number of new molecules discovered and put at the doctors' disposal tends to decrease.

First it is important to remind that the emergence of resistant bacteria is above all related to the massive use of antibiotics in human medicine and to bad hygiene conditions, in particular in communities (Ref. 68). Thus certain communities, where many individuals receive antibiotics, are particularly concerned: hospitals, but also day-nurseries, retirement homes…

The question of antibioresistance due to the use of antibiotics on farms raises different levels to be taken into account (Ref. 65):

- The use of antibiotics on farms can encourage the selection of bacteria resistant to antibiotics possibly transmissible to humans through food of animal origin. If these bacteria are pathogen common to animals and humans (salmonella…), the acquired resistances may obviously create problems. The emergence of resistances is very often due to bad use practices (in particular inappropriate dosage).
- Indirectly, antibioresistances acquired by bacteria present in animals could be transferred by different mechanisms (plasmids…) to bacteria present in humans.
- Finally, the potential effect of the presence of residues in food based on the selection, in humans, of resistant bacteria seems almost nil.

If jusutified concerns exist regarding the impact of the presence of residues on the emergence of resistance, no study has yet proved that the presence of residues in food has directly been the cause of an increase of resistances. **It is true that the respect of MRL keeps the residual contents to levels that have few chances of being at the origin of the selection of resistant bacteria** (even if the concentrations capable of inducing resistances are very low).

Study on the effects of antibiotic residues on inducing resistances.

For example, erythromycine induces resistances on staphylococcus aureus at optimal concentrations set between 10^{-8} and 10^{-7} M (Ref. 64). This corresponds, taking the mole mass of this antibiotic into account, to 73 to 730 micrograms/l. The lowest of these concentrations nevertheless remains superior to the MRL of antibiotics in milk, which is 40 micrograms/l.

Please note that for milk, the antibiotic detection methods used (microbiological methods) enable to select milks which residue content is "without effect" on bacteria considered extremely sensitive and, in that respect, makes believe the probability of inducing an acquired resistance is extremely low.