

## 3.2. Maximum Residue Limit (or MRL)

### The Maximum Residue Limit (MRL): the regulation basis

Shortly after the discovery of antibiotics, it appeared that the latter could have consequences both on processing (inhibition of ferments) as well as on human health. The question of risks related to the presence of residues has therefore been known for a long-time (Ref. 46, Ref. 39).

At first, certain countries proposed the absence of detectable residues (with the methods used at the time). Certain European texts thus presented the concept of “absence of residues” but set a limit at 5 ppb for penicillin in milk. This limit, unrelated to a risk analysis for humans, corresponded to the detection threshold of the tests used and the absence of effect on lactic ferments.

---

#### Définitions

**Ppb:** part per billion, i.e.  $10^{-9}$  or, for example,  $\mu\text{g per kg}$ .

**Ppm:** part per million i.e.  $10^{-6}$  or, for example,  $\text{mg per kg}$ .

---

The Maximum Residue Limit (MRL) is the maximum concentration of residues in a product (milk, meat, egg...) considered by the authorities as **without sanitary hazard for the consumer and without effect on the manufacturing processes**.

This limit must not be exceeded for food of animal origin. The means of applying this MRL at all levels of the industry are not defined by the regulation. Thus, for the dairy industry, the MRL concerns above all the milk delivered by the producer to the dairy factory and then, non-processed milk: collect tank-truck, raw milk sold to the consumer...

**It is impossible to directly apply this MRL to processed products (butter, cheese).**

### “Zero residue” and MRL concepts

The transition from the concept of “zero residue” to that of MRL became unavoidable by the development of analysis methods. Indeed, further to a treatment, the elimination of the molecule by the organism progressively tends towards zero but over very long periods. **The detection threshold was progressively divided by one hundred or a thousand with the development of the analysis methods.** With the available methods, the concept of “zero residue” de facto tended to make the use of many medicines impossible for economical reasons. The transition to the MRL concept became possible with the development in toxicology knowledge:

- Demonstration of the absence of effect at low doses (see further).

- Progressive acquisition of methodological knowledge allowing to perform assays guaranteeing the “no-observed-effect-level”.

### A compromise concept

The concept of MRL is particularly interesting as it is a compromise between consumers and producers. Without forbidding the use of medicines, it defines the rules of use in safe conditions (Ref. 50, Ref. 35).

### Very large differences between countries

Even if efforts have been made to harmonize the MRL at world level (under the aegis of WTO: [www.wto.org](http://www.wto.org) and the Codex Alimentarius), it must be acknowledged that the latter still strongly differs from one geographical area to another. Thus, due to these MRL differences, the same chlortetracycline-based medicine is granted, for a given species, a withdrawal time of 7 days in Canada and zero in the USA (Ref. 35).

### Molecule classification in Europe

Europe: the molecules are sorted according to their belonging to one of the appendices (European regulation n°2377/90/CEE).

**APPENDIX I:** list of substances for which maximum residue levels have been fixed in the food from target species.

**APPENDIX II:** list of substances for which it does not seem necessary for public health protection to fix a MRL. The measured concentrations never reach a dangerous value.

**APPENDIX III:** list of substances for which a temporary MRL has been fixed.

**APPENDIX IV:** list of substances for which it is not possible to fix a MRL (they are therefore “forbidden” molecules). The residues present a confirmed or suspected danger whatever the limit and to lift the suspicion, there is a lack of information.

## Toxicological MRL and bacteriological MRL

**Toxicological MRL** is set to ensure the consumer's safety. This concept includes all elements related to the molecule's toxicity in the short or long term and whatever the nature of the effects observed on the individual or his descendants.

**Bacteriological MRL** aims at guaranteeing the absence of antibiotic residue effects on human digestive flora. Whether this flora modification has an effect on the human being or not, the limit is taken into account independently.

### Toxicological MRL relies on the No-Observed-Effect-Level and on the Acceptable Daily Intake

MRL fixing relies on three essential concepts:

- Search for the **No-Observed-Effect Level (NOEL)** with different biological tests.
- From this NOEL and safety factors (of 100 and 1000): calculation of an **Acceptable Daily Intake (ADI)**.
- From this ADI and knowledge of the average food ingestion per inhabitant and the repartition in the different tissues and organs: final calculation of the MRL (milk, meat...).

---

#### Definitions

**NOEL (No-Observed-Effect-Level):** dose below which the effects by experience on animals are null.

**ADI (Acceptable Daily Intake):** estimated from the NOEL, dose which a man can ingest daily without risk.

---

From these concepts, the setting of a MRL is based on the studies carried out in toxicology and on specific studies related to residues. In concrete terms, it goes through the different steps presented hereafter.

### Toxicological MRL calculation from the ADI and average consumptions

- The MRL is defined from knowledge of the average consumption.
- The concept of MRL depends on the hypothesis taken for the average consumption.
- In Europe, the latter (closer to a maximum consumption) is for example of:
  - . 300 g. of meat, 100 g. of liver, 50 g. of kidney (if poultry, 10 g. of kidney and 90 g. of fat) or 300 g. of fish (muscle and skin),
  - . 1.5 litres of milk per day,
  - . 100 grams of egg,
  - . 20 grams of honey.

Theoretically, the MRL should vary for a population with a very different average consumption.

Sometimes, the question lies in the case of children, where the ingested quantity by kilogram of body weight is superior to that provided (for milk: 180 ml/kg instead of 25 ml/kg). However, the consumed milk content is very low in fat, which in most cases is where most residues are concentrated. Otherwise the safety factors taken into account include for most parts this particular case (reminder: safety factor of 100 or 1000).

## Toxicology studies

- Acute toxicity performed on 2 species one of which is not a rodent.
- Chronical toxicity.
- Medium term toxicity (in general 90 days) with, at the end of this time, examination of the animals and histological analysis of all the organs.
- Genotoxicity (for example "Ames" test) and, if necessary, carcinogenicity. If genotoxicity tests show an important effect, no MRL is generally granted.
- Reproduction toxicity and embryotoxicity.

### No-Observed-Effect-Level setting

These studies help define the "No-Observed-Effect-Level" which has no toxicological or pharmacological effect (acute toxicity, embryotoxicity, carcinogenicity...).

### Calculation of the Acceptable Daily Intake as regards to toxicology

A safety coefficient of 100, 1000 or more is applied to determine the ADI. The safety coefficient takes into account the transfer from animal to human and the population heterogeneity (age, sex, weight).

### Toxicological MRL setting

Based on this ADI and taking into account an individual's average consumption (60kg man whose consumption is described below), it is then possible to determine the MRL for each product. The MRL is therefore the limit which guarantees the ADI is respected.

### **Calculation of bacteriological MRL**

In the case of antibiotics, it is necessary to take into account the possible effects on human flora. Complementary studies are performed on a hundred bacteria strains from the human digestive tract in order to determine the No-Observed-Effect-level on this flora. These studies consist in searching the maximum dose with no effect on the multiplication of bacteria. It consists in cultivating separately each tested strain and exposing the latter to increasing concentrations of antibiotics. The effect considered as significant is that of the most sensitive strain. Once this MIC (Minimum Inhibitory Concentration) established, a safety coefficient of around 10 is applied taking into account the human digestive volume. This is called bacteriological MRL, coming as a complement to toxicological MRL.

### **Final MRL setting for the molecule**

The official MRL will be the lowest obtained between toxicological MRL and bacteriological MRL.

Experience shows that, for antibiotics, in most cases the bacteriological MRL is lower than the toxicological MRL.

### **MRL depending on the country**

The official MRL is defined for each country (USA) or group of countries (European Union). The values can vary quite relatively (see the tables hereafter).

### **Different withdrawal times for similar medicines**

Veterinarians are sometimes surprised by the different withdrawal periods they observe between different medicines, though similar, or, for the same medicine from one country to another. To understand these differences, it is necessary to know the history of implementation of both MRL and withdrawal times. Before 1992, each European country fixed their own MRL and at the time set the rules to determine the withdrawal periods. The first major reform was a common definition of Maximum Residue Limit for all countries inside the European Union. These MRL ease intracommunity food product exchanges with thresholds recognized by all countries. Further to the implementation of this regulation, the governments brought changes to their regulations. However, it turned out that the differences between countries on setting withdrawal times ended in differences in the actual withdrawal periods. In view of this situation, the European Union, as of 1998, on one hand defined the guidelines for setting withdrawal periods and on the other hand suggested the implementation of European registration procedures. A new step was taken again when it was required to fix the withdrawal time taking into account the residual dose on the molecule injection site. The succession of these events explains why veterinarians still have today medicines, which having obtained their market authorization at different times, and having been submitted to different regulations, therefore have today different withdrawal times. The major coordination effort at European level keeps going today. This effort will progressively lead in the years to come to the solving of what sometimes appears as a source of questioning for veterinarians.

table n°1 - MRL examples for milk in Europe, in the USA and for the Codex

Family	Molecule	Milk		
		MRL EU	MRL Codex	MRL USA
Beta lactam antibiotics	Penicillin G (Penethamate)	4	4	5
	Ampicilline	4		10
	Cloxacilline	30		10
	Oxacillin	30		50
	Cephalexin	100		
	Cefalonium	20		
Aminoglycosides	Neomycin	1500	500	150
	Gentamicin	100	200	30
	DHS et streptomycin	200	200	125
	OTC	100	100	300
	CTC	100	100	300
Macrolides	Spiramycin	200	100/200	
	Tylosine	50		50

table n°2 - MRL examples for pork meat in Europe, in the USA and for the Codex

Family	Molecule	Meat		
		MRL EU	MRL Codex	MRL USA
Beta lactam antibiotics	Ampicilline	50		10
	Amoxicillin	300		10
	Ceftiofur (+ marker)	1000	200	1000
Aminoglycosides	Neomycin	500	500	1200
	Gentamicin	50		100
	DHS and streptomycin	500	1000	2000
Tetracyclines	OTC	100	100	2000
	CTC	100	100	2000
Macrolides	Spiramycin	200	200	
	Tylosine	100		200

table n°3 - Alphabetical search of the main antibiotics used in veterinary medicine and their MRL

Molecule	Family	MRL for milk	MRL for pork meat
Oxolinic acid	Fluoroquinolones	(0)	100
Amoxicillin	Beta-lactam	4	50
Ampicilline	Beta-lactam	4	50
Bacitracin	Polypeptides	100	-
Cefacetil	Beta-lactam	125	-
Cephalexin	Beta-lactam	100	200 (bv)
Cefalonium	Beta-lactam	20	-
Cefapirine	Beta-lactam	60	-
Cefazoline	Beta-lactam	50	-
Cefoperazone	Beta-lactam	50	-
Cefquinome	Beta-lactam	20	50
Ceftiofur	Beta-lactam	100	1000
Chloramphenicol	Phenicol	(0)	(0)
Cloxacilline	Beta-lactam	30	300
Colistine	Polypeptides	50	150
CTC	Tetracycline	100	100
danofloxacin	Fluoroquinolones	30	100
DHS et streptomycin	Aminoglycosides	200	500
Dicloxacillin	Beta-lactam	30	300
Difloxacin	Fluoroquinolones	0	400
Doxycycline	Tetracycline	-	100
Enrofloxacin	Fluoroquinolones	100	100
Erythromycine	Macrolides	40	200
Flumequine	Fluoroquinolones	50	200
Gentamicin	Aminoglycosides	100	50
Kanamycin	Aminoglycosides	150	100
Lincomycin	Macrolides	150	100
Marbofloxacin	Fluoroquinolones	75	150
Nafcillin	Beta-lactam	30	
Neomycin	Aminoglycosides	1500	500
Nitrofurane / Furazolidone	Nitrofuranes	(0)	(0)
Novobiocin	Macrolides	50	-
OTC	Tetracycline	100	100
Oxacillin	Beta-lactam	30	300
Penicillins (benzylpenicilline)	Beta-lactam	4	50
Pirlimycin	Macrolides	100	100 (bv)
Rifaximine	Ansamycin	60	-
Spectinomycin	Aminoglycosides	200	300
Spiramycin	Macrolides	200	250
Sulfadiazine	Sulphonamides	100 for the total sulphonamides	100 for the total sulphonamides
Sulfadimethoxine			
Sulfadimidine			
Sulfadoxine			
Sulphonamides			
Sulphafurazole			
Sulphamerazine			
Sulphamethazine			
Sulphamethoxazole			
Sulphaquinoxaline			
Sulphathiazole			
Tetracycline	Tetracycline	100	100
Tiamuline	Pleuromutiline	-	100
Tilmicosine	Macrolides	50	50
Tylosine	Macrolides	50	100