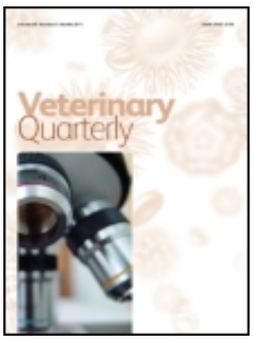

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Veterinary public health: Human health hazards associated with the administration of antimicrobials to slaughter animals

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HUMAN HEALTH HAZARDS ASSOCIATED WITH THE ADMINISTRATION OF ANTIMICROBIALS TO SLAUGHTER ANIMALS

PART I. AN ASSESSMENT OF THE RISKS OF RESIDUES OF TETRACYCLINES IN PORK

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Review Papers

SUMMARY

This article describes the assessment of consumer risks of residues of tetracyclines in slaughter pigs in the Netherlands. The assessed risks were toxic and allergic reactions, and the disturbance of the consumers' intestinal flora. Toxic and allergic reactions in humans and animals have only been observed at therapeutic doses, affecting between an estimated 1 in 5000 and one 1 in 140,000 individuals exposed. Residues of tetracyclines in pigs are closely associated with treatment with injectable formulations. Established Maximum Residue Levels (MRLs) do not reflect actual consumer risks in case a limit is violated incidentally. For example, when the established MRLs for tetracyclines in meat are exceeded with a factor 400, 40,000, and 200,000, respectively, the actual risk of an adverse drug reaction for the consumer following a single consumption of this meat is maximally 1 in 3 million, 1 in 300,000, and 1 in 8000, respectively. At the current estimated low levels of incidental exposure via pork, the annual risk of negative health effects for a random consumer is estimated at maximally 1 in 33 million. The annual risk that a temporary disturbance of the intestinal flora may also result in a facilitated infection with certain enteropathogens, such as *Salmonella* spp., is estimated at 1 in 45 million. It is concluded that the current microbiological risks of pork are greater than the risks of residues of tetracyclines as such, and that the control of the microbiological risks of pork should therefore be given first priority.

INTRODUCTION

The research project 'A Risk Assessment-Like Approach to the Modernization of Meat Inspection' was initiated to investigate which approach would enable the assessment and comparison of the microbiological and toxicological risks of the production and consumption of meat in the Netherlands. This would make it easier to set objective priorities regarding which risks should be controlled.

Human health hazards associated with the veterinary use of tetracyclines in slaughter pigs can be grouped into three categories: (I) toxic, allergic, mutagenic/teratogenic effects of residues in pork and edible by-products (1,2,6,7,26,29,38,69,73); (II) disturbance of the composition of the normal intestinal flora of individuals by the presence of residues (21,44,66, 69,77,84,85); (III) spread of microbial resistance to humans through the consumption of pork contaminated with resistant bacteria (12,26,29,44,84,85). The first two categories will be discussed here, the third in a companion paper (20).

The approach adopted for risk assessment in this study was based on a detailed description of the fate of the hazardous agent investigated throughout the entire production chain, from 'stable to-table'. This includes an account of all knowledge regarding the influence of factors affecting the presence or absence of the agent and of the health effects on animals and humans. The recorded data-set can be defined as a descriptive epidemiological model (3,15). A detailed account of the terminology used, how and why different kinds of models can be used, the actual construction of such models, the further uses of such models, and the limitations of the approach is given by Berends *et al.* (15).

The model for tetracyclines in slaughter pigs and pork was primarily built with data from the literature. These data were only included in the model if they were an estimate of a risk or if they could be used to estimate risks. Some of the data used have not been published in international scientific journals (2,4,5,8,9,13,30,37,41,42,62,78,79,80-82), or were verbally provided by an anonymous spokesperson of the Veterinary Public Health Inspectorate (10).

In the risk assessment models, health risks were 'standardized' as the expected frequency of new cases of disease, contamination, or a clinically apparent pathophysiological effect (i.e., the annual incidence). The influence of certain identified risk factors was mostly quantified by using the measure Odds Ratio (OR) and occasionally the Attributable Fraction (AF). The strenght of the associations was tested for signifigance with the Chi-Squared Test or, when appropriate, the Fisher Exact Test (11,50). The OR is the ratio between the odds of disease or contamination in the case that the factor is present or absent, and the AF is an estimate of the proportion of cases that is really caused by the factor being present in a particular (sub)population. When the OR of a factor is significantly larger than 1 it is a definite risk, and when it is smaller than 1 it works preventively.

In this paper the actual assessment of consumer health risks of tetracycline residues in slaughter pigs and pork were assessed according to the following scheme, a scheme that is common for a toxicological risk assessment (3,15,83):

(1) Hazard identification (also called hazard evaluation), i.e. the listing of all observed harmful effects of the agent of concern in animals or humans.

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Table 1. Documented side effects of tetracyclines in humans (1,2,6,7,25,38,69,73).

Side -effects	Remarks
Discolouration of teeth of children < 8 years, including hypoplastic enamel	Discolouration consists of complexes with calcium phosphate. Occurs in children < 8 years of age and/or in children whose mother was treated during the 4th-9th month of pregnancy or when lactating.
Deposition in bone tissue, including disturbed osteogenesis	Deposition consists of complexes with calcium phosphate. Tetracyclines are slowly released from the complexes, in contrast to the depositions in teeth.
Nausea and stomach burn, including ulcerations in the oesophagus	Result of direct irritation of the (gastric) mucosa.
Pseudomembraneous colitis and super infections	Serious complications because of proliferation of *Clostridium difficile* in the intestine. Much more common are super infections with *Candida albicans*.
Fatty liver degeneration	Predominantly seen as complication in pregnant patients. Predisposing factors are kidney disorders, malnutrition and severe infections.
Neutropaenia, leucopaenia, thrombocytopaenia, aplastic anaemia, haemolytic anaemia	Sometimes associated with endotoxin release of bacteria causing the infection following administration of the drug.
Benign cranial hypertension	Clinical symptoms disappeared following discontinuation of therapy.
Worsening of muscle weakness in patients with myasthenia gravis	Magnesium ions in the drug formulation turned out to be the cause.
Anaphylactic shock	Few descriptions. No deaths observed. Often preceded by allergic skin reactions, such as erythema and urticaria.
Asthma	Few descriptions. Preceding skin reactions also seen.
Tubulonephrosis	Associated with high contents of degradation products (anhydro forms) due to incorrect storage of the drug.
Worsened renal insufficiency	Few times observed in patients already suffering from the disorder. No noticeable effects when steady state plasma concentrations are kept below 16 mg/L.
Diabetes insipidus	Seen with demethylchlortetracycline.
Elevated ureum levels with normal creatinine levels	Probably due to disturbed protein synthesis in the eukaryotic host.
Photo-toxic dermatitis	Result of fluorescence of the drug after exposure of the skin to sun light (ultra violet light).
Urticaria, erythema, exanthema, contact dermatitis	Allergic reactions to the actual drug and/or components of the formulation.
Near-sightedness	Probably due to changes in light breaking properties of the corpus vitreum. Symptoms disappeared following discontinuation of therapy.
Pigmentation of skin, thyroid gland, cornea and lenses	Depositions of complexes with calcium and/or melanin.

(2) Dose-response characterization (also called dose-response evaluation), i.e. the determination of quantitative relationships between administered doses and the observed effects in animals and, if such data exist, in humans.
(3) Exposure characterization (also called human exposure evaluation), i.e. the determination of the sources, routes, quantities, and (active or inactive) chemical forms in which the agent of concern reaches humans.
(4) Risk characterization (also called risk evaluation), i.e. the combination of the three preceding steps, with the aim to assess the probability that random individuals, or random members of certain vulnerable population (sub)groups, will experience adverse health effects from the current levels and routes of exposure.

When the dose-response relationships was unclear, extrapolations were made under the assumption that a doubling of the dose would lead to a doubling of numbers exposed that show adverse reactions (3,83). Furthermore, the amounts of residues at injection sites or in tissues, were always estimated as high as (theoretically) possible, and the extrapolations of dose-response relationships were always based on the highest numbers of exposed individuals that showed an adverse reaction at a certain dose. This resulted in highly conservative estimates of the health risks involved. However, this made it also pointless to use confidence intervals or to do Monte-Carlo simulations.

AN ASSESSMENT OF HUMAN HEALTH RISKS OF RESIDUES OF TETRACYCLINES IN PORK AND EDIBLE BY-PRODUCTS

1 Hazard identification:

The tetracyclines are a group of chemically closely related substances that are widely used to treat bacterial infections in hu-

mans, animals and some fruits, such as pears, peaches, tomatoes and coconuts (6,7,71,74). At therapeutic doses they exert a mainly bacteriostatic activity against Gram-negative and positive bacteria (4,38,48,49,74). In prokaryotic organisms in particular, the substances act by binding reversibly to the 30S subunit of ribosomes, which ultimately disturbs the cell protein synthesis (6,7,48,49,71).

When kept dry or dissolved in organic solvents, such as propylene glycol, the tetracyclines are stable, however, when dissolved in water (as a salt) or exposed to (ultra violet) light, humidity, or heat, tetracyclines are rapidly degraded and lose their antimicrobial activity. One of these degradation products, the anhydro tetracyclines, are known to be toxic (6,7,38,49). Other degradation products, such as epi or iso tetracyclines, are not known to be more toxic than the parent drug. Furthermore, since these products do not bind to the 30S subunit of ribosomes they are inactive as an antimicrobial (6,7,38).

Documented undesirable side-effects of therapy with tetracyclines in humans are listed in table 1. Most frequently occuring side effects are a temporary nausea and mild stomach burning, caused by direct irritation of the gastric mucosa, which usually does not require discontinuation of therapy (1,2,6,7,25,38,69, 73). In addition, tetracyclines are not carcinogenic (4,6,7,38).

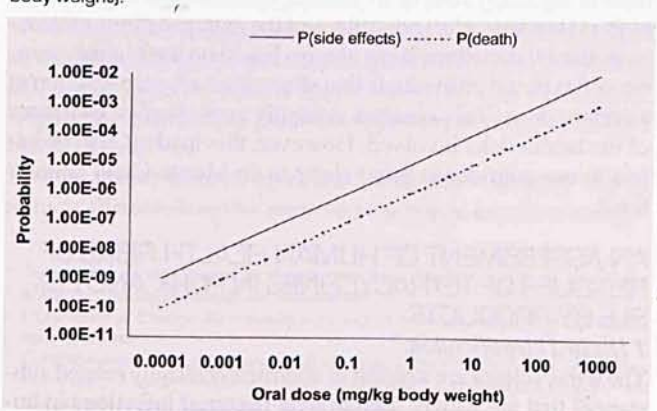
The effects of tetracyclines on the intestinal flora are important. A common effect of treatments is a superinfection of the gastrointestinal and/or urogenital tract with organisms that are intrinsically resistant to tetracyclines, such as *Candida albicans*, *Proteus*, and *Pseudomonas* spp. (4,38,73). Rarer, but much more to be feared, is pseudomembraneous colitis caused by *Clostridium difficile* (38,48,49,84). Another undesirable side-effect is the disturbance of the resistance to colonization of the gastrointestinal tract. This means that it is easier for certain enteropathogens, such as *Salmonella* spp., to colonize the intestine (13,16,20,21,23,24,33,70,84).

2 Dose-response characterization:

In laboratory animals the acute toxicity of tetracyclines is low. The lowest oral LD_{50} (the dose at which 50% of the exposed died) varied for mice between 2200 and 7000 mg/kg body weight (b.w.), and for rats it was 4800 mg/kg b.w. (6,7,25,48, 49). The oral LD_{50} for humans of about 60 kg can be extrapolated to be between at least several dozens to several hundreds of grams (25,83).

Side-effects of tetracyclines in humans are practically always observed after therapy with doses of about 20 mg/kg body weight or higher, and are often associated with certain characterictics of the patient, such as metabolic disorders (1,2,4,6,7, 28,38,48,49,69). The lowest (oral) dose of tetracyclines that can provoke allergic reactions is not exactly known. Moreover, the chemical structure of tetracyclines is such that they do not serve directly as an epitope or become an epitope after conjugation with other substances or molecules (1). Most of the reactions that have in the past been termed allergic are probably more associated with a biochemical reaction, such as phototoxic dermatitis, or were caused by other molecules in the pharmaceutical formulation of the product (see also Table 1) (1,38). The incidence of allergic reactions to penicillines administered in therapeutic doses is estimated to be between 1 in 2500 and 1 in 70,000 treated, and anaphylactic shock is estimated to be lethal in maximally 1 in 50,000 treated (38). The incidence of toxic and allergic reactions after therapeutic use of tetracyclines, however, is estimated to be at least twice as low (38). Therefore, it is assumed in this review that oral doses of tetracyclines, oxytetracyclines, or chlortetracyclines at a therapeutic level of 20 mg/kg b.w. may lead to noticeable side-effects in maximally 1 in 5000 exposed. Figure 1 is an extrapolation of this estimate, ranging from a dose of 0.1 µg/kg b.w. to 1 g/kg b.w. For example, at a dose of 1 µg/kg b.w. about 1 in 100,000,000 exposed may show side effects.

Figure 1. Roughly estimated maximum probability of side effects of various doses tetracyclines in humans (normal oral dose: 10-20 mg/kg body weight).



The anhydro degradation products, in particular, are held responsible for acute kidney damage following administration of incorrectly stored tetracyclines (38,72,74). There are no known data about the proportion of degradation products in formulations. Considering that (1) normal daily oral dosing of in total up to 20 mg/kg b.w. leads to steady-state plasma concentrations of 2 to 2.5 mg/L, (2) steady-state plasma concentrations in excess of 16 mg/L can cause kidney damage, (3) the percentage of degradation products in incorrectly stored formulations may vary between 1 and 50% of the total amount of tetracyclines, it can be inferred that the anhydro degradation products are a factor 10-100 more toxic than the parent drug.

Table 2 summarizes dose-response relationships between tetracyclines and the effects on the intestinal flora (2,24,27,28,33, 65). It appears that if adults are exposed to 1-2 mg/kg b.w., the composition of their intestinal flora or the prevalence of resistant bacteria is not altered. In animals, feed that contains less than 5mg/kg does not seem to have an effect either.

3 Human exposure characterization:

The use of teracyclines as a growth promotor is forbidden in the Netherlands and in most of the other EU member states. In the Netherlands, tetracyclines amount to approximately 50% of all the antimicrobials used for therapy and prevention of disease in pigs (22). Furthermore, up to 99% of all tetracyclines are used for mass medication of pigs, via the feed or drinking water, and the remainder are used for individual treatments with injectable formulations (22,30,31,51). The main substances used are oxytetracycline and chlortetracycline; tetracycline or doxycycline are seldom used. Other tetracyclines are not administered to pigs in the Netherlands.

The pharmacokinetics of oxy- and chlortetracycline, in particular, are very similar in man and pigs (35,51,52,56,57,61, 71,72). On average, 25% (10-30%) of an orally administered dose of the active compound is absorbed through the mucosa of the stomach, the duodenum, and the jejunum. Uptake in the colon is negligible. Tetracyclines are not metabolized by the host, and so significant amounts of chemical degradation products, and of the anhydro forms in particular, are not formed (1,72,73). Of the absorbed amount, about 80% is (passively)

Table 2. Lowest doses of tetracyclines that did or did not affect the intestinal flora or the selection of resistant bacteria.

Species	Dose	Remarks	ref
Adult human	50 mg per person per day	Continuous administration for 9 days did not lead to noticeable changes in numbers of resistant *E.coli*. Exogenous resistant *E. coli* of bovine origin did not succeed in colonizing the intestine or in passing on their plasmid-mediated resistance to the residential flora	24
Adult human	1 mg per person per day	No noticeable effects during 14 days of continuous administration	27
Human	40 µg/kg b.w./day	Proposed upper limit for life long continuous exposure	2
Dog	2 mg/kg in feed	No noticeable effects during 44 days of feeding	33
Chicken, mouse, pig	5 mg/kg or more in feed	Continuous administration leads to changes in proportions of certain aerobic species,. better feed conversion, and, at least in gnotobiotic animals, a smaller thymus and thinner intestinal wall	2, 28
Gnotobiotic mouse	6.5 mg/L drinkingwater	Colonization/selection experiment with continuous administration of the drug. At this concentration the number of plasmid PYD1 bearing *E. coli* rose from 10^4 to 10^5 colony forming units(cfu)/gram dropping	27
Gnotobiotic mouse	12.8 mg/L drinking water	Same result, but this time with plasmid PC1 bearing *E. coli* (all experimental groups were before the experiments exclusively colonized with *E.coli*, leading to about 10^9 cfu *E. coli* /gram droppings)	27
Gnotobiotic mouse	16 mg/L drinkingwater	Lowest concentration at which all the observed effects of the tetracyclines were also statistically significant	This study
Bacteria in general	0.25 MIC50	A quarter of the Minimal *(in vitro)* 50% Inhibiting Concentration (MIC50) is not supposed to affect microorganisms	2
Bacteria in general	0.125-0.20 MIC	At concentrations between 1/8 and 1/5 of the MIC50- value there were no observed effects on microorganisms	65
E. coli	0.25 mg/L growth medium	Lowest concentration of tetracyclines that could affect growth of fully sensitive cultures (MIC 50:1 mg/L)	27

excreted via the urine and 20% via the bile. Tetracyclines excreted via bile are partly reabsorbed (i.e., an enterohepatic cycle).The amounts that are reversibly bound by the skeleton and teeth are negligible in the framework of a risk assessment of the consumption of pork and edible by-products (35,51,52, 56,57,61,71,72). When humans are administered a daily oral dose of 1000 mg, the concentration in the excreted faeces is circa 500 µg/kg (48,49). The concentration in the contents of the lumen of the small intestine is about 250 µg/kg (27). The difference is due to concentration of the contents in the colon, as a result of absorption of water. In the intestines, about 20% of the unabsorbed dose of tetracycline is unbound, and therefore active as an antimicrobial. The rest is irreversibly bound by the contents of the intestine.

Residues of antimicrobials in pigs are strongly associated with parenteral administration, i.e., intramuscular or subcutaneous injections. Using data from Smeets *et al.* (82) it was calculated that in about 94% of cases the residue-positive pigs had been treated with injectable formulations (95% confidence interval (CI): 71-99%). The elimination half-life of parenterally adminstered tetracyclines in pigs is, depending on the pharmaceutical formulation and the site of injection, between 3 and 30 times longer than that of orally administered teracyclines (35,51,52, 56,57,61,63,64,71-77). Using data from Harbers (36), it was calculated that animals injected with antimicrobials tested positive 2.6 times more often at slaughter than orally medicated pigs (i.e., an OR of 2.6 for injectable formulations). Hard data about the incidence of injection sites and the quantities of antimicrobials actually present are virtually absent. The following could be used to make an estimate (original data not shown):

1. When the antemortem inspection categorizes animals as healthy, the postmortem inspection will on average detect only 20% of all the macroscopic lesions that are actually present in 1% or less of the animals (i.e., those lesions that have true prevalence of 1% or less) (14,36).
2. Harbers (36) found that postmortem inspection detected injection sites in 0.1% of nearly 10,000 slaughtered pigs that the antemortem inspection had categorized as perfectly healthy. The true prevalence of injection sites in clinically healthy slaughter pigs can therefore be calculated at 0.5% (i.e., five times 0.1%).
3. In pigs that are already considered as suspect by antemortem inspection the true prevalence of injection sites is on average 15% (55).
4. Pigs categorized by antemortem inspection as having localized abnormalities, such as an inflamed tarsal joint, amount to 1% of all pigs slaughtered in the Netherlands. Animals categorized as apparently suffering from a more generalized disease amount to about 0.5% of the total number of slaughtered pigs (5,8,9,14,36).
5. The total prevalence of pigs with injection sites that are not detected during meat inspection can therefore be estimated at maximally about 0.7 to 0.8% (i.e., 0.5%+0.15%+ 0.075%).
6. Not all visible injection sites will contain residues at the time of the slaughter. However, in about 30% of these cases the injection site will contain 0.1 to 100% of the original dose, thus causing a positive test result with the usually used microbiological screening test (8,10,55,56,59,61,68, 71,75,76,82).
7. In the Netherlands about 10% of the pigs that test positive

Table 3. Estimated current annual probability that a random Dutch consumer experiences clinically apparent health effects of one or more exposures to pork containing an injection site with tetracyclines (for calculations: see text).

Product category	Injection site containing maximally	Annual probability of side effects
Pork chops	4 mg	$2.2 * 10^{-11}$
	400 mg	$1.1 * 10^{-9}$
	2000 mg	$3.3 * 10^{-9}$
Filets	4 mg	$1.1 * 10^{-11}$
	400 mg	$5.5 * 10^{-10}$
	2000 mg	$1.7 * 10^{-9}$
Roasts	4 mg	$3.7 * 10^{-12}$
	400 mg	$1.9 * 10^{-10}$
	2000 mg	$5.7 * 10^{-10}$
All products together:		$7.4 * 10^{-9}$

for residues of antibiotics at the time of slaughter contain tetracyclines (10,36,82).

8. Considering points 5-7, an estimated maximum of 0.03% of all Dutch pigs that pass meat inspection will contain residues of tetracyclines.
9. The EU-prescribed monitoring for residues of antimicrobials in healthy slaughter pigs detects on average 0.22% (0.19-0.26) positive animals with a test that has a sensitivity of about 80% (8-10,36,42,43,54,55). The true prevalence of residue-positive animals will therefore be on average 0.28% (0.24-0.33%).
10. An estimated 95% of these animals have been treated with injectable formulations, and an estimated 10% of these contain residues of tetracyclines. Therefore, the data from the EU-prescribed monitoring schemes further substantiate the estimates made in points 1-8.
11. Because of the inhibition zones seen in practice, it can be roughly estimated that: (I) about 40% of the kidneys that test positive contain between 0.3 and 2 mg tetracycline/kg kidney tissue, (II) 30% contain between 2 and 12 mg/kg, and (III) 30% contain between 12 and, as a theoretically possible maximum, 65 mg/kg (8,10,37,58,59,60,68,71, 73,76).
12. (I) If a kidney contains up to 2 mg tetracycline/kg, the meat may contain up to 0.7 mg/kg and the liver up to 1.4 mg/kg; (II) if a kidney contains up to 12 mg tetracycline/kg, the meat may contain up to 4 mg/kg and the liver up to 8 mg/kg, respectively; (III) if a kidney contains up to 65 mg tetracycline/kg, the meat may contain up to 8 mg/kg and the liver up to 16 mg/kg (35,48,49,51,52,56,57,61,63,64,71-77).
13. Thus it can be estimated that on average (I) the meat and liver of 0.012% of pigs slaughtered may contain up to 0.7 mg/kg and 1.4 mg/kg tetracyclines, respectively; (II) the meat and liver of 0.009% of pigs slaughtered may contain up to 4 mg/kg and 8 mg/kg, respectively; (III) the meat and liver of 0.009% of pigs slaughtered may contain up to 8 mg/kg and 16 mg/kg, respectively.
14. On the basis of pharmacokinetical data, back-calculated corresponding maximum levels of tetracyclines at the injection site are then (I) 4 mg, (II) 400 mg and (III) 2000 mg, respectively (35,48,49,51,52,56,57,61,63,64,71-77).
15. Considering that in the Netherlands yearly about 6 million pigs are slaughtered for the local market, an estimated 1800 carcasses with residues may eventually reach the consumer (i.e., 0.03%). *Of these, an estimated 720 (i.e., 0.012%) will contain an injection site with maximally 4 mg tetracycline, 540 (i.e., 0.009%) with maximally 400 mg tetracyclines, and 540 (i.e., 0.009%) with maximally 2000 mg.*

During cold storage, the amounts of residues present in carcasses, meat or edible by-products do not change (67,69). Although heating, such as cooking or frying, leads to degradation of the parent compound, the toxicity of the formed products is the same as or less than that of the parent drug (6,7,40,67). The formed products include, for example, isochlortetracycline, α and β-apo-oxytetracycline, and epitetracycline. Furthermore, cooking does not lead to the formation of measurable amounts of anhydro-tetracyclines.

Also, the usual preparation of pork results in an average 10% loss in microbiological activity (67).

The absorption of the active compound from the intestine and tetracycline residues from meat may differ graetly. As noted previously, on average 25% of the pure compound becomes absorbed (range: 10-30%). Rats fed meat from slaughter animals treated with radioactively labelled tetracyclines, absorbed maximally 10% of the amount administered via the meat (47). However, when the meat was obtained from slaughter animals treated less less than 24 hours before slaughter, a few rats absorbed maximally 23% of the administered amount of labelled drugs. The same phenomenon is observed after feed medication, where on average 5%, but actually between 2 and 20%, of the drug administered via the feed is absorbed (52,72).

4 Risk characterization

4.1 Risks of clinically apparent pathophysiological effects

A consequence of the virtual lack of data about the prevalence of residue containing injection sites in slaughter pigs is that a variety of other data had to be combined to obtain a plausible estimate (see above). In the Netherlands, it is customary to inject antimicrobials in the neck of slaughter pigs. These necks are always processed, and it is highly likely that injection sites are noticed by slaughterers and butchers. Moreover, because cutting plants and butchers are financially compensated for meat with such deficiencies, there is no incentive for them to further process the often discoloured (and abnormal tasting) tissues. It is therefore estimated that of the 1800 injection sites that may reach the cutting plant or butchers' shop:

1. At least 95% of the 'acute' injection sites, i.e., those containing the full dose up to 2000 mg, are intercepted.
2. Minimally 90% of the 'subacute' injection sites, i.e., those containing up to 400 mg, are intercepted.
3. 85% or more of the 'chronic' injection sites, i.e., those containing up to 4 mg, are intercepted.

Considering the exposure characterization (see above), an estimated maximum of 108 sites containing 4 mg tetracycline (15% of 720), 54 sites containing 400 mg tetracycline (10% of 540) and 27 sites containing 2000 mg tetracycline (5% of 540) might actually be purchased by consumers.

Table 3 displays the estimated annual consumer risks regarding the consumption of injection sites that still contain tetracyclines. These calculations were based on the following additional data and assumptions (original data not shown):

1. In the Netherlands pigs' necks are normally used to produce 14 pork chops ('karbonade'), 8 filets ('lapjes') and 2 roasts ('rollade'). This means that, with an annual number of 6 million pigs that are brought onto the market, yearly 84 million chops, 48 million filets and 12 million roasts are produced.

Table 4. Estimated current annual probability that a random Dutch consumer experiences clinically apparent health effects of one or more exposures to pork and edible by-products that have passed meat inspection and that contain tetracyclines (for calculations: see text).

Tissue-type	Containing maximally	Annual probability of side effects
Muscle	0.7 mg/kg	$1.8*10^{-9}$
	4.0 mg/kg	$6.5*10^{-9}$
	6.0 mg/kg	$1.3*10^{-8}$
Kidney	2.0 mg/kg	$2.4*10^{-11}$
	12.0 mg/kg	$1.8*10^{-10}$
	65.0 mg/kg	$1.2*10^{-10}$
Liver	1.4 mg/kg	$1.0*10^{-10}$
	8.0 mg/kg	$3.5*10^{-10}$
	12.0 mg/kg	$5.4*10^{-10}$
All tissues together:		$2.2 * 10^{-8}$

Assuming a consumer population of 15 million pork-eating people, the average annual consumption per capita can therefore be estimated at 6 chops, 3 filets, and 1 roast.

2. Assuming that the injection sites are equally divided between chops, filets, and roasts, the probability that a random consumer purchases a pork product with an injection site containing 4 mg tetracyline is then $7.4*10^{-7}$, containing 400 mg $3.7*10^{-7}$ and containing 2000 mg $1.9*10^{-7}$.
3. The probability that an event with the small (independent) probability P(x) occurs once or more during n repetitions can be calculated as: $P_{(\text{once or more occurring})} = 1-(1- P(x))^n$. Considering the annual purchases, it can thus be estimated that, for example, the annual probability that a random consumer purchases, at least once a year, a pork chop with an injection site with 4 mg tetracycline is $4.4*10^{-6}$ (i.e., $P_{(\text{once or more occurring})} = 1-(1-7.4*10^{-7})^6$).
4. If all the abnormal tissue is cooked and eaten by the same person, and all the residues are absorbed, the probability that exposure to a dose of 4 mg would lead to clinically apparent effects in a 'standardized consumer of 60 kg' can then be estimated at $5*10^{-6}$ (i.e. 1 in 200,000; see also figure 1).
5. The ultimate probability that a random consumer would experience detrimental side-effects can therefore be estimated at $2.2*10^{-11}$ (i.e., $4.4*10^{-6} \times 5*10^{-6}$). The probability that someone would also die from this exposure can be estimated at least ten times lower (i.e., $2.2*10^{-12}$).
6. The summation of all calculated risks leads to a total annual risk of unwanted side-effects due to consumption of pork containing an injection site with residues of tetracyclines of $7.4*10^{-9}$. The risk of death is at least ten times lower (i.e., maximally $7.4*10^{-10}$). Furthermore, if it is considered that in practice only about 25% of the dose ingested via the meat will be absorbed, and that it is unlikely that abnormal looking (and tasting) injection sites will be consumed entirely, the risks calculated in this review are an overestimate.

Table 4 shows the estimated consumer risks of the consumption of pork and edible by-products with residues of tetracyclines.The following additional data and assumptions were used for the calculations (original data not shown):

1. Based on data from the Dutch Product Boards for livestock, Meat and Eggs, it is estimated that pork is purchased 'on average'about 150 times a year and is consumed in portions of 150 grams (wet weight before cooking) (13).
2. The probability that a random consumer purchases pork containing 0.7, 4 or 6 mg/kg tetracycline (see table 4) once or more times a year can therefore be set at 1.8%, 1.3% and 1.3%, respectively.
3. Pig kidneys and livers are not very popular items in the Netherlands and are mostly processed into cooked pork products, such as pâtés. Normally, this dilutes the concentration of residues. For reasons of clarity, however, it is assumed that an individual eats 'on average' 0.8 kidney (of approximately 250 g).
4. Similarly, it is assumed that an inidividual eats 6 portions of pigs' liver (of approximately 100-150 grammes) annually.

In conclusion, the total annual risk of clinically noticeable health effects in the Dutch consumer caused by consumption of pork and edible by-products and/or an injection site with residues of tetracyclines can be estimated at maximally $3*10^{-8}$ (1 in 33 million), on the assumption that the ingested amounts of tetracyclines are fully absorbed. If it is assumed that approximately 25% of the ingested amounts are absorbed, the annual risk for a random consumer is $7*10^{-9}$ (1 in 133 million). The risks of death can be estimated as maximally $3*10^{-9}$ and $7*10^{-10}$, respectively. However, it is highly likely that these risks are lower.

4.2 Risks of disturbance of the consumers' intestinal flora

Short-term steady-state concentrations of 12.5 mg/kg tetracyclines in the small and 25 mg/kg in the large intestines of human adults do not affect the composition of their gut flora (2,24,27). In slaughter animals, however, long-term administration of feeds with >5 mg tetracycline per kg does affect the composition of the intestinal flora and the prevalence of resistant bacteria (2, 20,28,33). For slaughter pigs, this means daily doses of about 0.1 mg/kg b.w. (52,72,73). This leads to a concentration in the contents of the small intestine of about 0.7 mg/kg and in the large intestine of 1.4 mg/kg, respectively. About 20% is not bound to the faeces and therefore active as antimicrobial. It is therefore assumed that these concentrations might have an effect in humans too.

Considering the above, it is estimated that for measurable effects on the intestinal flora a 'standardized consumer of 60 kg' should ingest daily about 2.5 mg tetracyclines or more (i.e., a dose of 0.04 mg/kg b.w.). In other words, when on average 150 grammes of meat are consumed, (daily) consumption of pork containing more than 16 mg/kg could therefore, have effects on the intestinal flora (i.e., 2.5 mg/150 g results in a 'microbiological tolerance limit' of 16 mg/kg).

The effects of a single exposures to small amounts of tetracyclines are difficult to estimate. In humans, however, the effects of a single exposure to a therapeutical dose of a broad spectrum antibiotic on the composition of the intestinal flora can be observed up to 48 hours later (21,23,85). The effects of 3-5 days of oral therapy can be measured for 5-7 days following the last administration of antimicrobials (13,15,23,24,70). Considering the exposure characterization and the calculated 'microbiological tolerance level' of 16 mg/kg b.w. for continuous consumption, it is inferred that the consumption of an injection site might have an impact on the composition of the flora, but that the consumption of organs and meat with the calculated maximum amounts of residues will have no noticable effects.

Based on the estimated non-detection rates of injection sites, annually up to 108 people might consume an injection site containing maximally 3.6 mg of tetracyclines, 54 people a site with maximally 360 mg, and 27 people a site with maximally

1800 mg after cooking, respectively. If all the drug from the injection site is released in the intestine, the concentration of unbound tetracyclines in the small intestine will be 0.2, 90 and 450 mg/kg, respectively, and in the large intestine 0.4, 180 and 900 mg/kg, respectively. Therefore, per year about 81 consumers are exposed to tetracyclines in an amount that might be enough to disturb their intestinal flora for 1-2 days. For a random consumer this is an annual probability of $5.3*10^{-6}$ (1 in 189,000).

The most important consequence of a disturbed intestinal flora is that the risk of colonization of the intestinal tract with a pathogen, for example, *Salmonella* spp., is increased (by about 5.5 times) (16,70). For a random adult consumer in the Netherlands, the average annual probability of becoming colonized with *Salmonella* spp. via food of animal origin is on average $3*10^{-3}$ (1 in about 300) (13,19). The probability that in the same period a person is exposed to both *Salmonella* spp. and an amount of tetracylines in pork that is enough to disturb the intestinal flora, so that the consumer becomes more easily colonized with the enteropathogen, can therefore be estimated at $2.2*10^{-8}$ (1 in 45 million). The risks of a colonization by other bacterial species, such as *E. coli* or enterococci, are of the same order of magnitude (20).

DISCUSSION AND CONCLUSIONS

A risk assessment is as accurate as the data upon which it is based. There is a paucity of adequate data. The complete lack of (international) data about actual amounts of residues in slaughter animals that pass meat inspection was very suprising (1-3,5,8-10,36,37,40,42,63,79-81). Moreover, pharmacological and toxicological data in the literature can seldom be used directly for a quantitative approach of actual consumer health risks of meat, because most of the data on, for example, observed undesirable side-effects of tetracyclines stem from clinical case reports. In addition, the quality of both the information and the sources is highly variable (6,7,38). If a future system of safety assurance of meat is to be based on risk assessments, it is necessary to monitor the amounts of residues that actually reach the consumer. Better accessible information about dose-response relationships is also needed. The cooperation of the pharmaceutical companies is, therefore, indispensible, because much of the data are classified.

A consequence of these limitations is that the assessments made are based on several assumptions. Though the assumptions were made as objectively as possible, and were mainly based on published information, their validity has yet to be proven. Furthermore, calculations were often based on observed or estimated extreme values. The amounts of residues at injection sites or in tissues, for example, were always assumed to be as high as theoretically possible, while dose-response relationships were always assessed conservatively. Consequently, it was pointless to use confidence intervals or to perform a Monte-Carlo simulations.

Nevertheless, even with these limitations it is concluded that currently the exposure of the (Dutch) consumer to residues of tetracyclines in pork seems to bear little risk. That the estimated risks of tetracyclines in pork are so small is, however, not very surprising. First, tetracyclines have been used safely in humans for over 40 years. Secondly, EU legislation concerning residues in food animals is primarily based on criteria that reckon with very wide safety margins, an exaggerated consumption of food of animal origin, and life-long continuous exposure to the substances (32,34,39,43,45-47,53,54).

It is also because of this last aspect that the established tolerance limits are not helpful in assessing any actual risks for the consumer when such a limit is exceeded incidentally. The established tolerance levels, also called Maximum Residue Limit (MRL), for tetracyclines in pork are 0.1 mg/kg tissues for uncooked muscle, 0.3 mg/kg for uncooked kidney and 0.6 mg/kg for uncooked liver. These limits ensure that lifelong daily ingestion of these products will not cause any noticeable pathophysiological response in the exposed. However, the estimated risks of exposure in practice appear to be considerably lower than is suggested by the magnitude an MRL can be exceeded. Table 2 illustrates this discrepancy between theory and practice. For example, an injection site with an assumed average mass of 100 gram contains tetracyclines in concentrations of up to 40 mg/kg, 4,000 mg/kg or 20,000 mg/kg. The MRL for muscle tissue in these cases is exceeded by up to 400, 40,000 and 200,000 times, respectively. However, in daily life consumers are only incidentally exposed to injection sites. The actual consequences of such an incidental exposure to the estimated maximal amounts of tetracyclines present in these examples are that about 1 in 3 million, 1 in 300,000, and 1 in 10,000 exposed individuals may show clinically apparent adverse drug-reactions, respectively.

Furthermore, the estimated current maximal annual risk for random Dutch consumers of an adverse drug reaction following exposure to injection sites containing tetracyclines is 1 in 35 million. In contrast, in The Netherlands the current annual risk of getting salmonellosis via pork is estimated to be on average 1 in 1600 (13,19). The consumer risk of getting salmonellosis via pork is thus at least 80,000 times greater than the current health risks of residues of tetracyclines at injection sites. Furthermore, it is at least 20,000 times greater than the combined risks of residues of tetracyclines in pork and edible by-products. Therefore, the current microbiological hazards of pork must be considered as more important than the current risks of residues of tetracyclines in pork and edible by-products. The control of the microbiological risks should thus be given first priority.

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HUMAN HEALTH HAZARDS ASSOCIATED WITH THE ADMINISTRATION OF ANTIMICROBIALS TO SLAUGHTER ANIMALS

PART II. AN ASSESSMENT OF THE RISKS OF RESISTANT BACTERIA IN PIGS AND PORK

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SUMMARY

Risks for the consumer regarding the acquisition of resistant bacteria and/or resistance genes via the consumption of pork are discussed. In general, *Salmonella* spp. and *Escherichia coli* that originate from animals do not easily transfer their resistance genes to the resident intestinal flora of humans. The prevalence of resistant *E. coli* in humans seems more associated with being a vegetarian (odds ratio (OR) 1.89) than with the consumption of meat and meat products. Other risk factors are treatment with antimicrobials (OR 2-5), becoming hospitalized (OR 5.93), or working in a health setting (OR 4.38). In the Netherlands, annually an estimated 45,000 people (0-150,000) become a carrier of resistant *E. coli* and/or resistance genes that ori-