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CARBADOX - AN EVALUATION

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ABSTRACT

This report evaluates the pros and cons of using carbadox, a feed additive for pigs that has two claims: (1) growth promotion, (2) prevention against swine dysentery.

Carbadox is effective in the prevention of swine dysentery. As a growth promoter, however, its effectiveness is marginal (if farming conditions are optimal).

Carbadox is considered to be a genotoxic carcinogen. In addition it exerts adrenal toxicity in target animals. The compound has significant dusting properties.

Applying good agricultural and veterinary practice, residue formation of carbadox (and its metabolites) in edible products is negligible. Hence there is no risk for the consumer.

The applications of carbadox have hardly any alternative.

The benefits of carbadox need to be weighed carefully against its disadvantage, i.e. the principle of avoiding the use of carcinogenic compounds. Unfortunately, actual research data regarding the exposure of workers in the feeding industry and farms working with carbadox, that are needed for such a risk estimation, are currently lacking.

SAMENVATTING

Dit rapport evalueert de voor- en nadelen van het gebruik van carbadox, een veevoederadditief voor varkens met twee effecten: (1) groeibevordering, (2) preventie tegen varkensdysenterie.

Carbadox geeft goede preventie tegen varkensdysenterie. Onder optimale bedrijfsomstandigheden is de groeibevorderende werking echter marginaal.

Carbadox wordt als genotoxisch carcinogeen beschouwd. Daarnaast is het toxisch voor de bijnier van het doeldier. De stof heeft de eigenschap gemakkelijk te verstuiven.

Onder de condities van goede agrarische en veterinaire praktijk zijn de residuen van carbadox (en metabolieten) in eetbare producten verwaarloosbaar, zodat de consument geen risico loopt.

Met betrekking tot de toepassingen van carbadox zijn er nauwelijks alternatieven.

Er dient een afweging gemaakt te worden tussen enerzijds de voordelen van het gebruik van carbadox, en anderzijds het principe dat gebruik van carcinogene stoffen vermeden dient te worden. Helaas ontbreken de voor deze afweging noodzakelijke actuele gegevens met betrekking tot de blootstelling van werknemers in veevoederfabrieken en bedrijven waar met carbadox (bevattend voer) gewerkt wordt.

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1 INTRODUCTION

Carbadox (methyl-3-[2-quinoxalinylmethylene]-carbazate-N^1,N^4-dioxide, Mecadox®) is a feed additive used in rearing pigs. It is primarily used as a growth-promoting agent, or as the case may be, to improve feed-conversion. Secondarily it is used as a preventive agent against anaerobic intestinal infections, in particular *Serpulina hyodysenteriae* (previously known as *Treponema hyodysentariae)* - swine dysentery or dysentery Doyle. Carbadox was introduced in 1971 (Gropp et al, 1971, 1972) and has been accepted as a means to improve growth without a working mechanism being claimed ('group K'). In the EU the substance is allowed for administration to young pigs in doses of 20-50 ppm (parts per million, mg per kg) in the feed from a few weeks before weaning until the age of 14 weeks. Then a withdrawal period of 4 weeks needs to be adhered to before the animal can be slaughtered (Schumer, 1990).

2 EFFECTIVENESS

- growth promotion

In the first period following the introduction of carbadox, claims were made of improvements in feed-conversion and thus in growth-promotion of up to 25 %. In later years, this percentage dropped to a feed-conversion improvement of 5 % at most. In all probability this phenomenon (in the first instance having a high growth-promoting effect, and then later a considerably reduced effect) has to be attributed to improved hygienic farming conditions and to the adoption of a feeding-regime better suited to the needs of the animals. In the early stages already, it was suggested that carbadox could have an 'insulin-type' of effect. Besides this, it has also been shown that there is a link between the growth-promoting effect and lysine concentrations in the feed: with a low lysine level in the feed, the growth-promoting effect of carbadox was optimal. In other words, carbadox has a lysine-saving effect (Gropp & Wagner, 1990: Gropp et al, 1990). Under optimal conditions it appears that the growth-promoting effect of carbadox is otherwise nil. (Nabuurs & van der Molen, 1989).

- prevention of swine dysentery

Using the dosages allowed, carbadox offers good prevention against anaerobic intestinal infections, in particular *Serpulina hyodysenteriae* (previously known as *Treponema hyodysentariae*), also known as swine dysentery or dysentery Doyle. Carbadox is, however, not effective against an infection that is already present: the protection is exclusively preventive. In spite of being widely used during the past 25 years, carbadox has so far shown no resistance to the above mentioned anaerobic diseases. Contrary to this, resistance has been noted to aerobic pathogens such as *Escherichia coli* (Kital et al, 1979; Ohmae et al, 1981; Droumev, 1983; Baumgartner et al, 1985; Holá & Sevcik, 1990).

- alternatives

As far as is known, the feed-additive olaquindox (chemically closely related to carbadox) has similar growth-promoting properties, although to a less effective degree. As a preventative against swine dysentery, olaquindox is here again clearly less effective: the MIC (minimum inhibitory concentration) for *Serpulina hyodysenteriae* is ten times higher than the MIC of carbadox for this pathogen (Vijfhuizen et al, 1988).

Tylosine has been accepted as a feed additive as well as an animal medication for the prevention and cure of swine dysentery. Tylosine is, therefore, both a prophylactic as well as a therapeutic remedy (carbadox is exclusively prophylactically effective).

Unfortunately, however, resistance to tylosine has been noted on a large scale: already in 1988 all 35 field isolates of *Serpulina hyodysenteriae* in The Netherlands displayed an insensitivity to tylosine (Vijfhuizen et al, 1988).

In Sweden, carbadox (and olaquindox) are no longer allowed as feed-additives. This has led, on the one hand, to its *de facto* but not monitorable use through prescription by veterinary surgeons, and on the other hand, for the benefit of growth-promotion as well as the prevention of swine dysentery, the application of (very) large amounts of zinc oxide - up to thousands of ppms in the feed (Best, 1996; Björnerot et al, 1996). The growth-promoting capabilities of zinc preparations are highly questionable; furthermore, hardly any literature can be found about the effectiveness of zinc as a preventative against swine dysentery. Zinc is an essential micro-nutrient, the absorption of which is physiologically regulated. Administration of larger than homeostatically applicable amounts will thus not result in greater absorption, but only lead to direct and almost always quantitative excretions. Regardless of the possible beneficial effects to the animal, the application of zinc might potentially create an environmental problem that could have serious repercussions more or less like the wide-ranging consequences of the earlier high use of copper in the pig sector.

Besides, the use of a preventative agent against swine dysentery is superfluous when pig-farms operate under SPF conditions (specific pathogen free), as is presently the case in Denmark.

3 METABOLISM

Studies on the metabolism of carbadox were done in rats, monkeys, and pigs with ^{14}C-carbadox, labelled in the phenyl ring or in the carbonyl group of the side chain. The metabolism of carbadox is characterised by a quick reduction of the N-oxide groups and the cleavage of the methylcarbazate side chain. The primary metabolite in the urine was quinoxaline-2-carboxylic acid, which was also excreted in conjugated form. In the tissues, up to 24 hours after the last administration, residues of carbadox,desoxycarbadox,quinoxaline-1,4-di-N-oxide-2-carboxaldehyde,andquinoxaline-2-carboxylic acid could be observed. At the same time traces of hydrazine were found, but these were taken as having only a short life-span, quickly metabolizing further (SCAN, 1980, 1984, 1986; JEFCA, 1990a, 1990b, 1991).

4 TOXICITY

The oral LD_{50}[1] in the male mouse is 2810 mg per kg of body weight, and > 2810 mg per kg of body weight in the female mouse. Following intraperitoneal administration, the LD_{50} in the mouse is 1050 mg per kg of body weight. In the rat the oral LD_{50} is 850 mg per kg of body weight. Based on these data carbadox can be classified as being 'moderately toxic' (SCAN, 1980, 1984, 1986; JEFCA, 1990a, 1990b, 1991).

The administration of carbadox for a period of 5 weeks to 5-week old pigs in dosages of 0 to 200 ppm in the feed leads to damage to the zona glomerulosa[2] of the adrenals in the dosage groups > 50 ppm, that even after 11 weeks saw no recovery. This went hand in hand with a significant decline of the aldosterone level[3] in the blood (van der Molen et al, 1988). In a similar experiment, it was noted that as a secondary effect, apart from the decrease of the blood aldosterone level, also the renin-angiotensin[4] system was affected: blood levels were significantly raised in all dosage groups (van der Molen et al, 1989).

The decline of the aldosterone level in blood as a consequence of the administration of carbadox was confirmed in *in vitro* experiments (Baars et al, 1988). In continued research, it could be established that not only the blood aldosterone level decreases but also the levels of glucocorticosteroids, corticosterone, and cortisol, while at the same time the secretion of progesterone and analogues increases (Jager et al, 1994; Jager et al, 1996). Thus, it can be stated that a substantial disturbance of the steroid balance occurs, which has the consequence that the animal no longer reacts to salt imbalances and stress. Although this could be favourable for feed-conversion under certain circumstances, for the most part it has of course serious physiological consequences in general.

Regardless of the experimental indications of the carcinogenicity of carbadox (see further on) there are recent indications that disturbances of the steroid balance, such as caused by carbadox

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1 The LD_{50} is the single dose after which 50 % of the experimental animals die within 24 hours. To illustrate the 'moderate toxicity' of carbadox: its toxicity is between that of lead (with a LD_{50} of 120 mg/kg) and that of common salt (with a LD_{50} of 4000 mg/kg).

2 The zona glomerulosa of the adrenal is defined as the cell layer between cortex and medulla. These cells produce aldosterone.

3 Aldosterone is one of the mineralocorticoid steroid hormones, responsible for the regulation of water and salt homeostasis of the organism.

4 Renin is a glycoprotein produced by the kidneys. It stimulates the release of angiotensin, a glycoprotein hormone produced by the liver. Angiotensin acts as a vasocontrictor and increases blood pressure. It is also involved in the regulation of the blood aldosterone level.

(decrease of circulating corticosteroids and an increase of circulating progestagens), can by itself increase tumour incidence (Zumoff et al, 1981; Labrie et al, 1987; Gomes et al, 1988; Najid & Habrioux, 1990).

In addition recent research has made clear that not only olaquindox has photo-toxic properties (which was already known earlier), but also carbadox exhibits a certain degree of photo-toxicity in exposed humans, or, as the case may be, photo-allergenicity (Dawson & Scott, 1972; de Vries et al, 1990).

Supplementary to this, it can also be mentioned that data gathered from a 3-generation study of rats and a development study of rabbits treated with quinoxaline-2 carboxylic acid do not lead to positive findings that could indicate teratogenicity and embryotoxicity of this end-metabolite (JEFCA, 1990a, 1990b, 1991).

5 CARCINOGENICITY

- carbadox

In several chronic feed tests with rats, a dose-dependent increase of benign and malign tumours was observed at dosages of $\geq$ 1 mg carbadox per kg of body weight per day. Dosages of over 25 mg per kg of body weight were extremely toxic, so that the experiments could not be continued further. In 14 out of 15 genotoxicity studies in various mammals and non-mammals, positive results were reported. On the basis of these findings it was concluded that carbadox is obviously genotoxic and carcinogenic (SCAN, 1980, 1984, 1986; JEFCA, 1990a, 1990b, 1991).

- desoxycarbadox

The chronic administration of desoxycarbadox in a study with rats also resulted in an increase of tumour incidence. In all dosage groups (5 to 25 mg per kg of body weight per day) the incidence increased, mostly in the liver, but also in the skin and mammary glands (SCAN, 1980, 1984, 1985; JEFCA, 1990a, 1990b, 1991). Although desoxycarbadox scored negative in most mutagenicity tests, positive findings were reported in the cell transformation test and the Ames test with liver microsomes from rats pre-treated with PCBs. On the basis of these results, it was concluded that desoxycarbadox has probably a greater tumorigenic potential than the parent compound, so that desoxycarbadox most likely contributes significantly to the tumorigenic activity of carbadox in rats. According to the JEFCA (1990a, 1990b, 1991), the contribution of desoxycarbadox to carcinogenicity is nevertheless limited because desoxycarbadox is a short-living intermediary metabolite that quickly converts into quinoxaline-2-carboxylic acid (however, see the section 'Residues' for findings that elaborate on this hypothesis).

- methylcarbazate

The available information concerning methylcarbazate, a side-chain metabolite of carbadox, was limited. With this in mind it was concluded that neither the chronic studies in rats, nor the genrally used mutagenicity tests had led to positive findings. In other words, these studies do not indicate any carcinogenic and/or mutagenic potential of methylcarbazate (SCAN, 1980, 1984, 1986; JEFCA 1990a, 1990b, 1991)

- hydrazine

The structure of carbadox suggests that hydrazine is possibly a further metabolite of methylcarbazate. On the basis of the known pharmacokinetic properties of hydrazine and its chemical reactivity, it was hypothesized that this metabolite would quickly get eliminated. Consequently, it was concluded that, in spite of its known carcinogenicity and mutagenicity, the contribution of hydrazine to the carcinogenicity (and toxicity in general) of carbadox would at best be marginal (JEFCA, 1990a, 1990b, 1991).

- quinoxaline-2-carboxylic acid

With respect to quinoxaline-2-carboxylic acid, none of the chronic studies indicated any suspicion of toxic, carcinogenic or mutagenic properties of this metabolite (SCAN, 1980, 1984, 1986; JEFCA, 1990a, 1990b, 1991).

- conclusions regarding carcinogenicity

The above data led both the SCAN (1980, 1984, 1986) as well as the JEFCA (1990a, 1990b, 1991) to the conclusion that the risk of the use of carbadox on the basis of the carcinogenicity of the parent compound and its primary metabolite desoxycarbadox was so small that its use (under certain conditions) could be allowed.

Since then, however, the opinion concerning the use of carcinogenic chemicals and suspected carcinogenic chemicals has evolved. In 1989 the RIVM (the Dutch National Institute of Public Health and the Environment) came to the conclusion that carbadox must be considered to be carcinogenic and genotoxic. In 1991, the Arbeidsinspectie (the Dutch Labour Inspectorate) put carbadox as a carcinogenic compound on the list of materials that must be registered. Likewise, on the basis of recommendations of the Gezondheidsraad (1979, 1988) (National Health Council of The Netherlands) and general EU-rules, one is generally of the opinion that the use of genotoxic carcinogenic materials should be avoided wherever possible or just should not be allowed.

6 RESIDUES

Administration of 55 mg ^{14}C-labeled carbadox per kg of feed for a period of 5 days to pigs with a body weight of about 30 kg resulted in a concentration of total carbadox residues in muscle tissues of 5 μg per kg 30 days after the termination of this feeding-regime. After 45 days the concentration was decreased to 3 μg per kg, and after 70 days the level was 2 μg per kg. In liver, the concentrations were 74, 20 and 13, and in kidney 15, 5 and 4 μg per kg, respectively. In fat tissues the concentrations were 2 μg per kg after 30 days, after 45 days 1, and after 70 days < 1 μg per kg. Other residue studies have lead to similar results. In the liver of pigs, very small amounts of unknown metabolites were perceptible, even after 7 or more days after the administration had been terminated. Through alkali hydrolysis of the liver these residues could be partly released and converted into quinoxaline-2-carboxylic acid (JEFCA, 1990a, 1990b, 1991).

Residues of quinoxaline-2-carboxylic acid were studied in pigs with a body weight of about 12 kg that had been fed over a period of 47 days with feed containing 50 ppm carbadox. Immediately after termination of this feeding-regime, the concentrations of quinoxaline-2-carboxylic acid in liver, kidney and muscle tissue were 345, 211 and 30 μg per kg, respectively. After 7 days the concentrations were 169, < 30, and < 30 μg per kg, respectively. In liver there still remained 83 μg per kg after 14 days, and 48 μg per kg after 21 days (JEFCA, 1990a, 1990b, 1991).

In the studies mentioned above, carbadox and desoxycarbadox were only measurable during the first 72 hours after termination of the administration of carbadox. It should be stressed, however, that before 1990 the detection levels were about 5 ppb (parts per billion, μg per kg) in plasma, urine, tissues and organs. It was, therefore, assumed that after 28 days the concentrations in edible parts would be negligible. Although two of the three residues of carbadox in edible parts of pigs are supposed to be carcinogenic (carbadox and desoxycarbadox), it was also concluded that applying good agricultural practice, carbadox could be used in dosages of up to 50 ppm in the feed, with the application of a withdrawal period of 28 days (JEFCA, 1990a, 1990b, 1991).

It was noted that the - otherwise non-carcinogenic - (end)metabolite quinoxaline-2-carboxylic acid can serve as a marker: this metabolite can be measured for at least 28 days in tissues and organs (JEFCA, 1990a, 1990b, 1991), although the detection limit in the 1980s was 30 μg per kg, which is not exceptionally sensitive.

Research with more sensitive analysis methods has on the one hand confirmed the concentrations noted during and after the administration of carbadox to pigs, but on the other hand it has now

become clear that the biological half-life of the metabolites desoxycarbadox and quinoxaline-2-carboxylic acid in liver and kidneys is higher than was first noted. The research in question comprised 75, 3-week old pigs, that were administered 50 ppm carbadox in the feed for a period of 94 days. After cessation of the adminstration the animals were slaughtered at regular intervals. Already one day after termination of the administration no residues of the parent compound could be seen up to a level of 2 μg per kg. Desoxycarbadox dropped to 1 μg per kg in the liver after 14 days, in the kidneys after 7 days, and in the muscle tissue after 3 days. Three days after termination of the administration the level of quinoxaline-2-carboxylic acid in muscle dropped to 2 μg per kg; the MRL of 30 μg per kg in liver and kidneys was, however, only reached after 4 to 5 weeks (Baars et al, 1990).

In a recent experiment test mature pigs were given 50 ppm carbadox in the feed for a period of 7 days. Eight hours after terminating this regimen, no residues of parent compound and metabolites were demonstrable, measured up to a level of 1 μg per kg (Keukens & Tomassen, 1995).

Very recently a similar study was carried out to research possible residue formation as a result of carry-over[5] of feeds. A number of pigs received feed with a carbadox concentration varying from 0.5 to 5.4 ppm. The animals were subsequently slaughtered after withdrawal periods of 0, 3 and 12 hours. In the group given 5.4 ppm carbadox in the feed, the carbadox-residue in meat after a 3 hour withdrawal period was 1 μg per kg, after a 12 hour withdrawal period the concentration had dropped to < 0.5 μg per kg. In the livers and kidneys of these animals, no carbadox above the level of 0.5 μg per kg was found. After a 3 hour withdrawal period 3 μg per kg desoxycarbadox was noted in the meat, while the desoxycarbadox-concentrations in livers and kidneys were 2 and 25 μg per kg, respectively. After a 12 hour withdrawal period the concentration of desoxycarbadox in meat dropped to < 1, in the livers to 1, and in the kidneys likewise to 1 μg per kg. In the lower dosage groups no carbadox (to the level of 0.5 μg per kg) and desoxycarbadox (to the level of 1 μg per kg) was seen (Keukens, 1997).

Although the risk of residues of carbadox and/or desoxycarbadox in meat, assuming normal use of carbadox and observance of the prescribed withdrawal period, is thus virtually nil, this is less certain for liver and kidney.

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[5] The preparation of feeds with additives can result in contamination of the feed that is prepared after an earlier feed preparation. This phenomenon is called 'carry-over' and occurs particularly in using high dosages of additives and if the mixing and compressing devices and their respective pipes have not been cleaned properly. This contamination can also occur if the silo and/or the feeding troughs are not completely empty before they are filled with new and 'clean' feed.

7 DUSTING PROPERTIES

During the application of carbadox-containing feeds as well as during the production process of carbadox-containing concentrates, premixes and feeds, there is a certain risk of exposure for humans. Such exposure could take place through direct skin contact, through inhalation of the dust matter generated during the production and/or feeding process, through skin contact with the excretory products of the animals treated, and through residues in animal products. With respect to the second mentioned possibility, within the EU the requirement has been set that samples of concentrates in the analysis according to the Stauber-Heubach procedure (Stauber & Beutel, 1984) may not generate more than 0.1 μg carbadox in the dust.

In the beginning of the 1980s, studies were done in workers involved in the preparation of a 50 ppm carbadox-containing feed from a 10 % premix, and then administering this feed to pigs. Emphatically no measures were taken to prevent dust-formation during the process. The actual exposure of humans as well as pigs was measured with filters for a period of 15 days. On the average the 24-hour inhalatory exposure appeared to be 50 μg carbadox per kg of body weight. Unfortunately the report does not make clear whether the results regard an exposure during 24 hours or a 24-hours average on the basis of (for example) an 8-hour working day.

Experimental research has further shown that in a number of randomly taken samples, the requirements concerning dusting potential are often not fulfilled. In 25 samples in which the concentration of carbadox varied from 0.5 % to 10 % it appeared that the amount of dust released, determined according to the prescribed method, diverged from 0.34 to 14374 μg, with an overall average of 2818 μg and a median of 1610 μg (Aerts & Roozendaal, 1985). At the same time preliminary results showed that pretreatment of the concentrate to improve the dusting properties has no effect on the dusting potential of the premixes prepared from this concentrate (Aerts & Roozendaal, 1985). Even if it is accepted that, thanks to more hygienic farming measures, the risk of exposure during the production process has been reduced, then there still remains the risk of exposure when the dust-filters used in the production process are changed. As far as is known no research regarding this problem has been undertaken.

In order to allow reliable conclusions with respect to the risks of dusting for workers in animal feed plants and for people working with carbadox (whether or not added to feeds) renewed and more in-depth research regarding the dusting properties of carbadox is strongly recommended.

Starting from the requirements stipulated by the EU with respect to the maximum allowable concentration of carbadox in dust, as determined with the Stauber-Heubach method, an estimate can be made of the theoretical exposure. The Stauber-Heubach method (Stauber & Beutel, 1984) measures the amount of carbadox generated in dust-form from a (premix) sample of 50 g. This sample is placed in a standardized dust-generating drum equipped with a filter box, and rotated for 5 minutes while 4 l air per minute are drawn through the drum and filter. The dust collected in this way therefore originates from 20 l air, and should according to the EU-regulations not contain more than 0.1 μg carbadox, equalling 5 μg per m^3. Assuming a working period of 8 hours and a breathing volume of 7 litres per minute (resting state), an average adult person will be inhalatory exposed to 7 x 60 x 8 x 0.005 = 16.8 μg carbadox per working day, or 0.28 μg per kg of body weight per 8-hour working day. On a yearly basis (200 working days) this leads to a total inhalatory intake of 3.36 mg.

8 CONCLUSIONS

1. Although carbadox has certain growth-promoting properties for pigs (and possibly also for other farm animals), its beneficial effect is marginal (if not al all absent) if hygienic farming conditions are fully optimal, and optimal feeds and optimal feeding-regimes are used.

2. Carbadox is a good preventive (not curative) agent applicable in the prevention of infections by *Serpulina hyodysenteriae* (previously known as *Treponema hyodysenteriae*), the anaerobic micro-organism that causes swine dysentery.

3. Carbadox and its metabolite desoxycarbadox are considered to be genotoxic carcinogenic substances. In addition phototoxicity and photo-allergenicity have been observed.

4. The residues of carbadox and desoxycarbadox in edible porcine products are, with consideration to the rules, negligible in meat after 4 weeks, and in livers and kidneys after 6 weeks. Hence under conditions of Good Agricultural/Veterinary Practice the risk of exposure to carcinogenic residues of carbadox for the consumer of pork products is negligible.

5. The dusting properties of carbadox in concentrates, premixes and prepared feeds may led to exposure of people working in feed plants or working with carbadox (whether or not preparing feeds themselves) on farms. Workers in the animal feed sector where carbadox is handled thus are at risk of exposure to this compound.

6. Alternatives for carbadox are (1) olaquindox (indications: growth-promotion and prevention against *Serpulina hyodysenteriae* infections), and (2) tylosine (indications: prevention against and therapy in *Serpulina hyodysenteriae* infections).

   Olaquindox belongs to the same chemical family as carbadox: olaquindox is also suspected to be carcinogenic, above all the dusting properties are similar to those of carbadox. As a preventive agent against the above-mentioned infections it is clearly less effective than carbadox, besides this it is also phototoxic.

   Tylosine is not only effective as a preventive but also as a curative drug, but has already generated resistance.

   Operating pig-farms under SPF-conditions make the use of preventive agents against swine dysentery superfluous.

7. The pros and cons of carbadox, i.e. its benefits as a prevention against swine dysentery vs. the general principal that the use of carcinogenic compounds should be avoided, needs to be weighed carefully against each other.

   The only risk groups are the workers in the feed industry and the workers in agricultural practice involved with carbadox and/or carbadox-containing feeds.

   However, to allow a reliable estimate of these risks, up-to-date research data about the actual exposure of these people in their working environment are needed. At present such data are lacking.

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