

Opinion of the Scientific Committee for Animal Nutrition on possible risks for the consumer, the animal and the users (operators) from the use of Carbadox and Olaquinox as Feed Additives, 10 July 1998

**TERMS OR REFERENCE (JUNE 1997. REVIEWED AUGUST 1997 AND SEPTEMBER 1997)**

The Scientific Committee for animal nutrition is requested to re-evaluate the authorisations of carbadox and olaquinox, and to answer the following question: In view of the information provided to the Commission is there a risk for the consumers, the animal and the users (operators) by the use of quinoxaline-N-dioxides carbadox and olaquinox?

**BACKGROUND**

Carbadox [methyl-3-(2-quinoxalinylyl methylene) carbazate-N1, N4 -dioxide] was first admitted to Part E (other additives) of the Annexes to Council Directive 70/524/EEC concerning additives in animal feedingstuffs<sup>1</sup>, by the Eighth Commission Directive 74/378/EEC<sup>2</sup>. The Community authorisation (Annex I) was granted by Council Directive 87/316/EEC<sup>3</sup>. A new definition of carbadox was adopted by Commission Directive 90/110/EEC<sup>4</sup>. The approved conditions for use appear under table 1.

Olaquinox [2-(N-2' (hydroxymethyl) carboamoyl)-3-methylquinoxaline N1, N4 -dioxide] was first listed under part F of the Annex II (national authorisations), by the Sixteenth Commission Directive 76/933/EEC<sup>5</sup>.

- I. The Community authorisation was granted by Council Directive 87/317/EEC. The conditions of use of carbadox were modified in February 1996 by Commission Directive 96/7/EC<sup>6</sup>. The approved conditions for use of olaquinox appear under table 2.
- II The SCAN expressed its favourable opinion of the use of carbadox and olaquinox in the following reports:
  - A. Report of 6 July 1978, on the use of carbadox in feedingstuffs for pigs<sup>7</sup>;

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<sup>1</sup> Concerning additives in feedingstuffs (OJ No L270, 14/12/70, p.1), as last amended by Council Directive 96/51/EC amending Directive 70/524/EEC (O.J. No. L235, 17/9/96 p.39)

<sup>2</sup> Of 1 July 1974 amending the Annexes of the Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs. (O.J. No. L199, 22/7/1974 p.13),

<sup>3</sup> Of 16 June 1987 amending Directive 70/524/EEC concerning additives in feedingstuffs in respect of Carbadox. (O.J. No. L160, 20/6/1987 p.32)

<sup>4</sup> Of 19 February 1990 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs. (O.J. No. L67, 15/3/90 p.44)

<sup>5</sup> Of 1 December 1976 amending the Annexes to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs. (O.J. No. L364, 31/12/1976 p.18)

<sup>6</sup> Of 21 February 1996 amending Council Directive 70/524/EEC concerning additives in feedingstuffs (O.J. No. L51, 01/3/1996 p.45)

<sup>7</sup> Reports of the scientific Committee for Animal Nutrition, Second Series (1980) Report 6918 EN. Catalogue N° CDE-NK-80-002-EN-C (p.7).

- B. Report of 8 July 1981 on the use of olaquinox in the feedingstuffs for pigs;
  - C. Report of 7 July 1982 on the use of carbadox in feedingstuffs for pigs (second report)<sup>8</sup>;
  - D. Report of 3 May 1984 the use of olaquinox in feedingstuffs for pigs (second report)<sup>9</sup>;
  - E. Report of 18 November 1992 on the possible implication of olaquinox in cases of photoallergic contact-dermatitis developed in pig-farmers.
- III The CEAS study on “*The impact on animal husbandry in the European Community of the use of growth promoters in animal feed*” (see under references) raised concerns about the use of carbadox and olaquinox. These concerns addressed:
- A. the genotoxicity and carcinogenicity potential for the users of these molecules;
  - B. the weak adverse effects on target animals;
  - C. the impossibility to set an ADI because of lack of a marker residue;
  - D. the phototoxicity for users;
  - E. the fact that, as these molecules are “generics” the formulation is less suitable.
- IV The Scientific Conference on the use of growth promoters in meat production (see under reference) concluded also that a re-evaluation of the Quinoxaline-N-dioxides should be considered, because
- A. carbadox and olaquinox possess genotoxic and/or carcinogenic properties;
  - B. appreciable amounts of residues are found in treated animals;
  - C. ADI values cannot yet be established although their restricted use in pigs only during the first four months coupled with the specified 28-day withdrawal period should lead to negligible residues at the time of slaughter and minimise the risk of exposure for consumers;
  - D. it is questionable whether the use of genotoxic and carcinogenic feed additives is acceptable.
- V. The Federal Republic of Germany, in its communication of 14. February 1997 has invited the services of the Commission to re-evaluate the authorisations of carbadox and olaquinox, and in discussing this, during the meeting of the Standing Committee for Feedingstuffs<sup>10</sup> on 24-25 February 1997, several Member States gave their full support to the German request, and the Standing Committee decided to proceed to a re-evaluation of the quinoxaline-N-dioxides by examining

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<sup>8</sup> Reports of the Scientific Committee for Animal Nutrition, Fourth Series (1984) Report EUR 8769. Catalogue N° CD-NK-83-010-EN-C) (p.82)

<sup>9</sup> Report of the Scientific Committee for Animal Nutrition, Fifth Series (1986) Report EUR 1041EN. Catalogue N° CD-NK-86-003-EN-C, (p.11.)

<sup>10</sup> Council Decision 70/372/EEC of 20 July 1970 (O.J. L170, 3/8/70 p.1)

the new elements arising since the first authorisation<sup>11</sup> concerning the possible risks for consumers operators and animals due to the use of carbadox and olaquinox.

- VI Sweden provided on 19 March 1997 (SCAN/97/49) a list of references on Quinoxaline-N-dioxides followed on 10 April 1997 by an extended list including photocopies of scientific literature (SCAN/97/63).
- VII The firm CRAFT, on behalf of the firms manufacturing olaquinox as a generic feed additive, has submitted for examination by SCAN the results of further studies on the safety for users of olaquinox.
- VIII On 18 July 1997 the Dutch Ministry of Agriculture, Environment and Fisheries announced to the Commission the prohibition of carbadox in application of Article 11 of Directive 70/524/EEC on grounds that:
- carbadox has been found to be genotoxic and carcinogenic;
  - there is a serious risk for workers in the feed industry;
  - there is no reason for the use of carbadox under Good Agricultural Practice in animal husbandry;
  - and that preventive measures at community level are necessary in the short term in order to safeguard users and workers in the feed industry from any harmful substances.
- IX A report drawn up by the Rijks-Kwaliteitsinstituut voor land-en tuinbouwprodukten - Dienst Landbouwkundig Onderzoek entitled "Carbadox - an evaluation" was presented to the Commission and joined to the Dutch request. This document is claimed to contain detailed grounds for establishing that carbadox should no longer be approved as a feed additive.
- X. The firm Pfizer provided the SCAN members on 12 September 1997 with a file with a review of the Safety of Mecadox (carbadox) feed additive.

## **OPINION OF THE COMMITTEE**

### **1. Introduction**

Carbadox and olaquinox have been approved feed additives since 1974 and 1976 respectively.

Since then SCAN's evaluations of carbadox have been published as Opinions in 1978 and 1982 (Background, refs I and II). Its toxicologists further considered the safety of carbadox in 1988 in preparation for receiving a United Kingdom delegation to DG VI on the matter of worker safety. No further studies in experimental toxicity, metabolism, consumer or worker safety have been performed by the sponsor since 1988.

Olaquinox Opinions were issued in 1981 and 1984. In 1992 SCAN reported on photoallergy in olaquinox-exposed workers (Background, refs I and II). No further studies in experimental toxicology, consumer or worker safety have been performed by

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<sup>11</sup> Sixteenth Commission Directive 76/933/EEC of 1 December 1976 amending the Annexes to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs. (O.J. No. L364, 31/12/1976 p.18)

the sponsor since. Additional information on metabolism was provided to JECFA in 1994.

For carbadox and olaquinox the pig is the only approved target species. For both, use is permitted up to 50 mg/kg of complete feedingstuff for pigs up to 4 months of age and a 4 week minimum withdrawal period is required.

Both substances were reviewed again in 1990 at the 36th JECFA, the WHO/FAO Joint Expert Committee on Feed Additives. Olaquinox was again considered at the 42nd JECFA in 1994 in the light of additional metabolic studies. Despite the less than ideal safety profiles of the substances, resulting in requests for further information and certain constraints on approvals, on none of these occasions have these expert committees advised against their further use.

Notwithstanding, the Federal Republic of Germany has requested in its letter of 14 February, 1997 a re-evaluation of both carbadox and olaquinox and the Standing Committee for Feedingstuffs has proceeded with this by considering new elements concerning the possible risks for consumers, operators and pigs which have arisen since the 1976 authorisation. SCAN will follow the same course.

It would appear that the use of carbadox is proscribed in Denmark under national legislation governing carcinogens properties (SCAN 97/80).

A Netherlands ban on carbadox under Article 11 of Directive 70/524 was communicated to the Commission on 18 July 1997.

## **2. New elements**

It is important to emphasise that, with the exception of an additional study of the metabolism of olaquinox, the company data reviewed on the several occasions by SCAN and JECFA was the same. With the passage of time advances in test design and conduct have taken place. These do not, however, of necessity render older studies invalid for safety assessments. What has changed is that SCAN, in addition to its overriding concern for consumer safety, now adds further weight to worker safety and environmental matters. JECFA evaluations focus solely on consumer safety.

The “new elements” comprise summaries of the earlier evaluations and highlight the adverse properties of the drugs. In addition, there is new open literature information on the metabolism and kinetics of carbadox and olaquinox, on their phototoxicity, on their target animal safety and on microbial resistance. Finally, companies, sometimes not the original sponsors, have performed new studies on worker exposure to both additives.

## **3. Assessment of the new Elements**

As the points of concern in the various documents which gave rise to Question 91 show considerable overlap, this section is structured by concern rather than by source. The source is indicated by reference number for each point of concern.

### 3.1 CARCINOGENICITY (refs 1, 2, 3, 7, 8)

#### 3.1.1 Carbadox

While no tumours were found in a 2-year monkey study at doses up to 25 mg/kg b.wt./day, the hepatotoxicity and tumorigenicity and carcinogenicity of carbadox in 4 long term studies in rats established the liver as the primary target at 25 mg/kg b.wt./day (malignant transformation in 3 of 13 rats). The NOAEL for carbadox for nodular hyperplasia in the rat was established at 1 mg/kg b.wt./day. More importantly, its metabolite, desoxycarbadox induced carcinoma of the liver in all exposed rats at 25 mg/kg b.wt./ day. The major persistent metabolite of carbadox, quinoxaline-carboxylic acid (QCA) was negative for tumorigenicity in 3 studies in rats and 1 in mice at up to 100 mg/kg b.wt./day, the top dose tested. The carbadox side-chain metabolite methyl carbazate, although itself found non-tumorigenic in 2 rat studies at up to 10 mg/kg b.wt./day (the top dose tested), can generate hydrazine, which, by mouth has caused lung and liver tumours in mice and rats.

Both a 2 year rat and a 7 year dog relay toxicity studies, in which liver (rats, 10% of diet) and pork (dogs, 200g/day) from pigs fed at 4x the user rate of carbadox (without withdrawal period) was fed at 10x the expected human consumption rate, were free of histological abnormality.

**Comment** Carbadox, probably via its metabolite desoxycarbadox, is a rodent carcinogen<sup>1</sup>. The negative methyl carbazate studies support the view that any hydrazine formed from it is free of carcinogenicity at intakes which greatly exceed those of consumers exposed to carbadox residues.

The predictive utility of rodent liver tumours for man is often questioned by toxicologists in particular as for Carbadox when associated with hepatotoxicity and regenerative hyperplasia.

#### 3.1.2 Olaquinox

In 4 studies in rats and 3 in mice no evidence of carcinogenicity was found. A small increase in adenomas of the adrenal gland was reported in one rat study, but this was not sufficient to increase the total of animals with tumours. One mouse study showed small increases in adenomas (adrenal, lung and ovary) in top dose males.

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<sup>1</sup> Minority opinion of Prof. J. Leibetseder: "According to SCAN Document / 98/24 "Draft Commission Directive amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition", page 23, top 2.5. Carcinogenicity, "a carcinogen bioassay should generally be carried out in at least one rodent species." There is no necessity to carry out the bioassay in more than one rodent species. If this bioassay in one rodent species shows carcinogenicity it must, therefore, be assumed that the substance is carcinogenic. In principle, carcinogens should not be used as feed additives. "

**Comment** Olaquinox is not a carcinogen in rats or mice, but has increased benign tumours in one species, the mouse, in only one sex and only at the highest dose tested 54 mg/kg b.wt./day<sup>2</sup>.

### 3.2.GENOTOXICITY (refs 1,2,3,5,7,8)

#### 3.2.1 Carbadox

Positive findings occurred in 10 microbial and 3 of 4 mammalian tests, both in vitro and in vivo. By contrast, desoxycarbadox was negative in 14/18 microbial assays (including 6 host-mediated) and 2 of 4 mammalian tests in vitro. QCA was negative in an Ames test and in human lymphocytes in vitro. Methyl carbazate was negative in microbial systems and in human lymphocytes in vitro. Hydrazine was positive in 2 of 2 bacterial tests and in mouse lymphoma cells.

**Comment** Carbadox is genotoxic<sup>3</sup>.

#### 3.2.2 Olaquinox

In 14 microbial tests, olaquinox gave positive findings. In mammalian tests it was positive in 1 of 1 in vitro and 12 of 18 in vivo tests addressing various endpoints, including one weak positive in Chinese hamster spermatogonia. It does not, however, bind to DNA and several mammalian positive results were obtained at near toxic dose levels or with olaquinox whose purity differed from that of the original sponsor's product.

**Comment** Olaquinox is genotoxic<sup>4</sup> and possibly a germ cell mutagen. It is noted that no evidence to support the latter possibility was found in the mammalian, multi-generation reproductive toxicity test.

The predictive utility of genotoxicity tests for rodent carcinogenicity is far from absolute and little is known of their predictive value even for genotoxicity in man. While it is unlikely that further genotoxic substances would be developed for any class of product, that property, while having impact on the labelling, is not of itself regarded as sufficient to require the removal of existing chemical products from their markets.

### 3.3 "APPRECIABLE AMOUNTS OF RESIDUES ARE FOUND" (refs 2,3)

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<sup>2</sup> Minority opinion of Prof. J. Leibetseder: "Olaquinox is a rodent tumorigen. Tumorigens should not be used as feed additives."

<sup>3</sup> Minority opinion of Prof. J. Leibetseder: "In principle genotoxic substances should not be used as feed additives."

<sup>4</sup> Minority opinion of Prof. J. Leibetseder: "In principle genotoxic substances should not be used as feed additives."

This potentially worrying but unspecified statement arose at the Scientific Conference on the Use of Growth Promoters in Meat production. Responding to it allows a comment on the metabolism and fate of the drugs.

### 3.3.1 Carbadox

The compound is extensively metabolised to yield essentially the same urinary metabolite profile in rats and monkeys as in pigs (13 of 15 metabolites), validating the predictive value of the rat long term toxicity studies for other species. Urine plus faecal label accounts for 88.2% of an oral dose of ring-labelled carbadox at 50mg/kg feed within 72 hours. No N- oxides were present in urine and no carbadox was present in faeces. The rapid metabolism of carbadox is reflected in the presence only of QCA in pig liver 24 hours after a single dose of labelled carbadox. Free methyl carbazate-derived hydrazine, was detected in pig urine only after dosing at 7 mg/kg b.wt. (i.e. about 3 x user level). After 14 days, 0.1mg/kg carbadox equivalent radioactivity remained in pig liver. With side-chain labelling, 0.1-0.34 mg/kg carbadox equivalent radioactivity remained in liver at 5 days. In neither case was this very low-level residue fully characterised but some labelled carbon was shown to have been incorporated into physiological substances. Neither carbadox nor desoxycarbadox formed part of the residue, however.

The 1988 metabolism studies fed <sup>14</sup>C ring-labelled carbadox at 55 mg/kg diet to pre-conditioned 30 kg pigs (n=5m and 5f) established total radioactivity levels in liver (44.7±27.0), kidney (14.5±4.9), muscle (6.7±2.5) and fat (<2) µg/kg carbadox equivalents at 30 days of withdrawal. A repeat study also used identically treated 30 kg pigs (n=10) and again showed a progressive decline in residue levels in the 4 sampled tissues from 30 to 70 days of withdrawal. On this occasion the 30-day liver residue was 74±30.5 µg/kg, but the other tissues were essentially as before. The only metabolite present at all sampling times in a preliminary total residue study was QCA. The QCA content of the samples from both of the pig studies (n=20) was established. Only in liver, at concentrations of 9.3±6.7, and 18.9±10.4 µg/kg carbadox equivalents, was its presence clear at 30 days. Fat was not assayed in this study. The overall mean percentage of total residual radioactivity which was QCA at 30 days of withdrawal was 24.4%.

The absence of carbadox and desoxycarbadox from blood, liver, kidney and muscle of pigs (n=4) after 72 hours of withdrawal has been independently confirmed by liquid chromatography (MacIntosh et al, 1985). The following Dutch studies also essentially confirmed the earlier findings. Carbadox had declined to a level of 1 or 2 µg/kg after 24 h, quinoxaline carboxylic acid in muscle by 3 d (but achieved the JECFA MRL of 30µg/kg in liver only after 4-5 weeks), and desoxycarbadox in liver by 14 d (Baars et al, 1990). QCA was the only entity detectable at 30 days of withdrawal. A later study found no residues of carbadox or its metabolites after 8 h at a limit of 1µg/kg (Keukens and Tomassen, 1995).

**Comment** Because residues were detectable at 14 days, they were in that sense “appreciable”. However, the concentration present (even had it been confirmed by the subsequent study) was very low and in that sense could not be considered “appreciable”. Neither feed additives (Council Directive 70/524/EEC) nor animal medicines have to achieve zero residues in order to be approved, as the existence of the Council Regulation

2377/90 for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin makes plain. Of importance here is that the kinetics and the metabolism of carbadox, as well as the toxicity of its individual metabolites have been well and extensively studied. The early studies established that beyond 24 hours after dosing, none of the carcinogenic entities remain identifiable in pig tissues. Subsequent open literature studies cited above have not been quantitatively consistent but none have yielded results which suggest that the 28 day withdrawal period was unsafe for the consumer. Unusually, several metabolites have been subject to separate safety studies. From these, the safety of the major, long-lived metabolite QCA has been established. This information, plus the unusually long mandatory withdrawal period should provide for the consumer freedom from concern over carcinogenic residues.

### 3.3.2 Olaquinox

SCAN previously reported (refs ii 9,15) that rapid and extensive absorption followed by excretion into the urine characterises the fate of oral olaquinox in rat and pig. At user levels, muscle, liver and kidney residues were below the limit of detection of olaquinox (0.05mg/kg) by day 2. Around 70% of an oral dose in the pig is excreted unchanged in the urine accompanied by 16% as mono-N-oxides with 3 probable carboxylic acid derivatives accounting for the balance. Later studies confirmed these findings and completed the characterisation of the metabolites.

Olaquinox was reviewed by the 36th and 42nd JECFAs (1990 and 1994). These reviews confirmed the SCAN assessment of olaquinox metabolism and residue kinetics. In addition, methyl quinoxaline carboxylic acid (MQCA) was established as the marker residue, with muscle as the target tissue because the concentration relationship of MQCA to other metabolites was not established for liver or kidney. Radiolabel depletion from tissues was linear and there appeared to be no bound residues. By 28 days of withdrawal in long term studies at 25 and 100mg/kg in feed, porcine tissues were free of intact N-oxide structures, i.e. those responsible for the mutagenic effects of olaquinox. A new method which is quantitative to 0.5µg/kg was available for monitoring the assigned MRL ("based on good practice in the use of veterinary drugs") of 4µg/kg muscle. Further information on MQCA in liver and kidney was requested for 1996.

**Comment** Residues of olaquinox can in neither sense of the word be appreciable following 28 days of withdrawal. Presumably, therefore, the comment 3.3 did not apply to olaquinox.

### 3.4 WEAK ADVERSE EFFECTS ON TARGET ANIMALS (refs 1,7)

Both additives have caused field episodes of severe toxicity including deaths when included in pig diets at accidentally high (i.e. unauthorised) concentration. The narrow safety margin of both additives emphasises the need strictly to observe the approved conditions of use<sup>5</sup>. Effects mediated via adrenal cortex depression or damage were seen

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<sup>5</sup> Minority opinion of Prof. J. Leibetseder: "Nevertheless a number of cases of intoxication in practice are reported. A risk for target animals obviously exists."



both in the field episodes and in laboratory studies. Similar effects were later shown to be present at approved inclusion levels but were weak and inconsistent. In production units such levels rather than causing impairment of production indices, allow their enhancement.

#### 3.4.1 Carbadox

Slight histological change in adrenal zona glomerulosa cells was seen in 6-week Dutch studies at inclusion levels as low as 25 and 50 mg/kg of piglet diet. At these levels the lesions were completely reversible. The authors considered that their findings indicated adrenally-mediated impairment of fluid and electrolyte balance<sup>6</sup>.

#### 3.4.2 Olaquinox

As for carbadox except that effects were not consistently dose dependent.

**Comment** The effects of both products in pigs are much the same at the same exposure levels. The weak effects seen at user levels would not be judged as adverse from the standpoints of condition and performance while the corresponding clinical biochemistry findings were difficult to interpret, including the corticosteroid measurements. The facts that adrenal and other endocrine effects were seen in rats, but always at higher doses and a longer duration of exposure than those effective in the pig and were absent from a 2 year study in monkeys at up to 20 mg/kg/day (the highest dose tested), suggest the possibility of a special sensitivity of the porcine adrenal to both additives. While it is plain that both additives are toxic to pigs at dietary inclusion levels greater than those authorised, the significance of the observations and findings at authorised levels remains unclear.

### 3.5 NEITHER ADDITIVE HAS AN ADI (refs 2,3)

#### 3.5.1 Carbadox

JECFA selected QCA as the marker residue, established MRLs and considered the residues after 28 days of withdrawal to be acceptable for consumers, but did not allocate an ADI. When MRLs have been established by other means, the absence of an ADI, although unconventional, is without practical regulatory effect.

#### 3.5.2 Olaquinox

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<sup>6</sup> Minority opinion of Prof. J. Leibetseder: "At user level not only histological changes were observed, but also gross lesions were seen in pigs receiving 50ppm. The main features were retarded growth, deshydration with dry contents in the intestine, especially in the colon and findings suggestive of pica. After 10 weeks of administration changes were found at 25 ppm dosage and higher. In the 25 and 50 ppm group half of the pigs had hydropic changes of the glomerular zone (E. J. Van Der Molen: Pathological Effects of Carbadox in Pigs with special emphasis on the Adrenal. J. Comp. Path. 98, 55-67, 1988)."

In the case of olaquinox, JECFA has renewed an earlier decision that the residues were temporarily acceptable, has again declined to set an ADI on the grounds that metabolic data were insufficient and has required information on its nominated marker residue in liver and kidney. Finding itself unable to set an ADI is also a JECFA device for encouraging firms to provide missing data. However, an MRL for muscle of 4µg/kg olaquinox-derived residues measured as methyl quinoxaline carboxylic acid (MQCA) was assigned.

**Comment** The “new element” ignores the fact that Directive 70/524/EEC does not call for the identification of MRLs for feed additives. Further, it implies that without an ADI, residues are unacceptable, which is contrary to the SCAN Opinions and to JECFA decisions on carbadox and olaquinox. While it is conventional first to establish an ADI and from that to derive MRLs, that is not the only route by which an MRL can be established, nor is it always the sole determinant of the concentrations adopted as MRLs. The MRL of 30 µg/kg liver for carbadox (measured as QCA) established by the FDA on 19 March, 1998 (Sundlof, 1998) was selected because it ensures, using a linear extrapolation method on the results of carcinogenicity studies, that meat residues are free of carcinogenic risk.

The MRL of 4µg/kg olaquinox residues in muscle, measured as 3-methyl quinoxaline-2-carboxylic acid, was established because its quantitative relationship to olaquinox and its other metabolites was known and it is compatible with the amounts known to be present in muscle when, as expressed by JECFA, “olaquinox is used according to good practice in the use of veterinary drugs.”

### 3.6 PHOTOTOXICITY AND PHOTOALLERGY (refs 3,5)

These toxicities are not specifically required to be investigated in pre-marketing studies on feed additives. The information available has arisen following the post-marketing exposure of some workers in pig production. SCAN is aware of the problem and, has recognised that although severe when it occurs, that it is of low prevalence and preventable.

#### 3.6.1 Carbadox

The new elements contained no reports of such reactions to carbadox in people.

#### 3.6.2 Olaquinox

The 1985 and 1986 reports of 2 cases in Italy involved unprotected workers who hand mixed pig feed. The feed mill regulations now in place were designed to exclude the possibility of such avoidable exposures to feed additives. Further these cases predate the current anti-dusting specifications for olaquinox. The 1996 German report covers cases accumulated over 6 years (1987-1993) and lists 15 cases in pig farmers, 2 of which had contact with finished feed and 13 had contact with a 1g/kg concentrate<sup>7</sup>. Of these, 12

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<sup>7</sup> Minority opinion of Prof. J. Leibetseder: "Even with finished feed phototoxicity and photoallergy were observed. In some of the EU countries pigs are fattened mainly with corn cob mix (75 %) and a

were habitual and 2 occasional home mixers and only 1 wore gloves prior to sensitisation. Eleven patients used product which did not carry the required label warning against the generation of dust. Many of the patients had dermatitis before presenting with olaquinox photoallergy. Regrettably, as dusting is formulation dependent, no information is given as to the origin(s) of the additives. It is noteworthy, however, that the originator's patent on olaquinox expired in 1985, followed by a decline in market share with obvious implication for product identity within the German case report.

**Comment** The evidence of clinical cases implicates olaquinox, but not carbadox in the claims of those whose comments lead to this re-evaluation. Importantly, nearly all of the patients had failed to use protective clothing and they had practised hand-mixing. While instances of photoallergy and phototoxicity have occurred, it seems inappropriate to cite this in evidence against olaquinox when so many of the affected workers had not observed safe practice in the handling of a known sensitiser. The in vitro study which showed a plausible chemical route to possible sensitisation for both additives is of unknown value as a predictor for skin disease in humans (ref 4).

### 3.7 SERIOUS RISK FOR WORKERS IN THE FEED INDUSTRY (refs 5,7,8)

This mainly concerns workers exposed to carbadox during its production, or to the manufacture of carbadox as an additive or to the additive in use before or during feed assembly. The concern is proper because, as for the farm worker, exposure is to parent carbadox and not, as for the consumer, to trace amounts of a known non-carcinogenic metabolite. Those who change air filter mats in feed plants are cited as at special genotoxic or carcinogenic risk following possible exposure by the dermal and/or inhalatory routes.

#### 3.7.1 Carbadox

The possibility for absorption by workers if exposed to carbadox in factory or farm must exist. Animal tests show that <0.1% of 100mg held to the skin for 24 hours is absorbed. Human skin is a more efficient barrier than rat and rabbit skin and the particle size of the originally approved formulation is such as to make percutaneous absorption highly unlikely. The animal tests show that carbadox is well absorbed across the mucous membrane of the gut. Hence, if not absorbed by the lung, carbadox, if inhaled, is likely to be absorbed following mucociliary clearance and ingestion. Studies on dust exposed workers and non-supplemented control pigs in production units revealed the absence of QCA from urine samples, however (ref X).

#### 3.7.2 Olaquinox

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supplementary concentrate (25 %) with higher concentrations of feed additives mixed at farms. The risk of phototoxicity and photoallergy for workers, therefore, does exist and cases are reported."

While no specific claim was made for olaquinox in this category, the expectations must be as for carbadox. However, olaquinox applied to the skin of 2 volunteers for 48 hours was not detectable in urine samples.

**Comment** Existing, enforceable good manufacturing and working practices should exclude these risks and are a matter for surveillance and enforcement at the level of incorporation of additive into pre-mixtures and of pre-mixtures into feeds as well as during the process of feeding. A recent study (ref 5) implied failures in these areas. See also Section 3.6, 3.8 and 3.9

### 3.8 ... PREVENTIVE MEASURES ... ARE NEEDED ...(ref 7)

This relates to the risks outlined in 3.7.

#### 3.8.1 Carbadox

Directive 70/524/EEC specifies already a maximum permitted rate of formation of dust from the anti-dusting formulations whose performance was assessed in the approval process of both additives. A specific exposure avoidance warning is required on the packings of both products (ref I). See also the comment in section 3.9.

#### 3.8.2 Olaquinox

As above.

**Comment** The call for short-term measures to be taken at Community level seems not to have taken into account the existing Commission provisions for avoiding worker exposure. See also 3.9.

### 3.9 THE GENERICS PROBLEM (refs 1,3,5)

The CEAS Report in which this issue was raised was written in 1991. Its point was that while the worker safety aspect of the Directive 70/524/EEC approval for carbadox and olaquinox had been based on the assessment of the original manufacturers formulations, the patents on both additives had long since lapsed. Olaquinox and carbadox of various origins are now on the market in additional copy products, none of which has been subject to assessment by the Commission. This situation is legal provided that the copies comply with the specifications in Directive 70/524EEC. There is in the “new elements” recent data which suggests that some samples studied did not comply with the Annex-specified anti-dusting criterion (ref 5).

**Comment** The Commission was advised by SCAN in its 1982 Opinion (refs ii, 13) of health risks from un-evaluated formulations which might be poor copies of the originals. This was rectified by Council Directive 96/51/EC which beginning 1 April 1998 will place both carbadox and olaquinox in an Annex in order to link the authorisation to the person responsible for putting them into circulation. This will require proof that copy

products at least meet the specifications of composition and performance of the authorised originals. Those which do not may not be sold after 1 Oct, 1999.

Worker safety has also been regulated in by Directive 95/69/EC which addresses exposure in the establishments, farms included, in which additives, including carbadox and olaquinox, are required to be incorporated into animal feeds by specifying equipment, protection and enforcing compliance through inspection and monitoring.

For both products for which data was supplied to SCAN for this review compliance with the Stauber-Heubach dusting test was supported by data.

### 3.10 GOOD HUSBANDRY CAN REMOVE THE NEED FOR CARBADOX (ref 7)

When carbadox was first approved it was on the basis of its growth promotional properties. It later became apparent that the compound was also effective in preventing swine dysentery. It is now the case that the substance is used in pig production in some regions in order to achieve both benefits<sup>8</sup>. The proposition that good husbandry could remove the need for carbadox has to be supported by evidence. The RIKILT-DLO document supporting the Dutch ban on carbadox states that “the applications of carbadox have hardly any alternative.” It goes on to state that “in NL it remains fully effective and free of resistance problems in *S. hyodysenteriae*.” However, the document quoted only one reference in support of its claim that the efficacy of carbadox as a growth promoter is nil when husbandry is optimal. That reference described a toxicity study, not one designed to measure the efficacy of carbadox on production indices under commercial circumstances.

Conventional pig production can be maintained without carbadox only if other substances of comparable effectiveness remain available. For the growth promotional aspect other authorised antimicrobial feed additives have the same effect<sup>9</sup>.

### 3.11 SHOULD GENOTOXIC AND/OR CARCINOGENIC FEED ADDITIVES REMAIN IN USE (refs 2,3)<sup>10</sup>

Although it is understandable that many would respond in the negative to this question, the only scientific contribution proper to such a general question would be to provide on a case by case basis the quantitative risk assessment necessary for the appropriate Risk Analysis. The SCAN evaluations of these hazards in the cases of carbadox and olaquinox presented in Sections 3.1, 3.2, 3.3, 3.4, 3.6, 3.7, 3.8, and 3.9 and are discussed

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<sup>8</sup> Minority opinion of Prof. J. Leibetseder: "Carbadox is used to prevent swine dysentery. This is not in accordance with the guidelines for feed additives. Only substances for preventive measures which are listed are allowed as feed additives. Carbadox is not listed as feed additive for prevention or therapy."

<sup>9</sup> Minority opinion of Prof. J. Leibetseder: "Carbadox is registered as a growth promoter, not as a prophylactic measure against swine dysentery. The effect of growth promoter can be obtained by other feed additives. The prophylactic effect must be replaced by drugs."

<sup>10</sup> Minority opinion of Prof. J. Leibetseder: "In principle such substances should not be in use because risk assessment always gives only a certain probability, but not an absolute safety. Because there is no absolute need for growth promoting feed additives and because other performance enhancers without these undesired properties are available genotoxic and /or carcinogenic substances should not be used as growth promoting feed additives."

in Sections 4 and 6. Also appropriate to SCAN would be to provide to the risk-benefit consideration component of risk analysis, information which specifies the efficacy-determined part of the overall benefit.

**Comment** The SCAN response to this large question is given in the following sections

#### **4. Human Safety/Risk**

The issue of human safety can be approached by deciding whether SCAN's previous safety assessments of carbadox and olaquinox should be re-considered in the light of the foregoing 11 points. It depends also on the evidence which establishes the expected level of exposure under the circumstances of concern. The more obvious of these circumstances are dietary exposure of consumers and workplace exposure for those involved in product manufacture and use.

In its previous opinions (refs ii, 5,9,13,15 and 30) SCAN has not established ADIs for carbadox or olaquinox, apparently because the parent molecules were absent from animals at slaughter after 28 days of withdrawal. It did, however, express its view that the ingestion of the low-level, derived residues remaining after 28 days of withdrawal would not be harmful to the consumer. Furthermore, based on measurements of real exposure levels and the requirement for anti-dusting properties, it was considered that the risk of adverse effects for exposed workers would be negligible.

##### 4.1 THE CONSUMER (refs 2,3,4)

###### 4.1.1 Carbadox

While carbadox and its metabolites desoxycarbadox and hydrazine are proven rodent carcinogens, the consumer is not exposed to these entities because of their relatively short persistence in pig tissues following withdrawal of the supplemented feed. This evidence is then reinforced by the added safety measure of requiring the exceptionally long withdrawal period of 28 days to be applied in pig production. The most persistent metabolite, *quinoxaline carboxylic acid, the form in which the consumer would be exposed to residues of carbadox*, is a non-carcinogen, was free of toxicity in a 2 year study in rats from which a NOEL of 100 mg/kg b.wt. day, the highest dose tested could be derived. It is the JECFA and FDA nominated marker residue and its concentration in pig liver, the tissue of its longest persistence, is of the order of 20 µg/kg at 28 days of withdrawal.

Eating 100g liver provides an intake of 2µg and 300g muscle would yield <1µg QCA. These amounts fall within the JECFA MRLs of 30µg/kg of liver and 5µg/kg for muscle for carbadox-derived residues. Compared with the rat NOAEL of 100 mg/kg for QCA, this provides a large safety margin. To this could be added the re-assurance that the 2 and 7 year relay toxicity studies in rats and dogs respectively fed daily with zero-withdrawal pig tissues to achieve 10 times the expected daily human exposure to carbadox residues were free of evidence of carcinogenicity and other adverse effects.

SCAN endorses the JECFA and the FDA safety evaluations of carbadox for the consumer and endorses also the MRL concentrations assigned.

#### 4.1.2 Olaquinox

Olaquinox is proportionally much less extensively metabolised than carbadox and is therefore excreted unchanged to a very much greater extent (about 70% of a single dose in urine in 24 h). Because they have not been subject to individual evaluation, less is known of the safety of the metabolites of olaquinox. However, the main metabolites in the pig have been shown to be non-mutagenic. Further, the long term predictive toxicology studies will have evaluated simultaneously the toxicity of olaquinox and all of its metabolites. There is no parent olaquinox in the carcass after 28 days of withdrawal and bound residues are absent or negligible. The multigeneration reproductive toxicity assay yielded a NOAEL of 2.5mg/kg b.wt./day.

Liver at 28 days of withdrawal contains olaquinox equivalent to <5µg/kg radioactivity, kidney <10 µg/kg and muscle 4µg/kg.

These concentrations would yield a residue intake of 0.5µg from liver, 0.5µg from kidney and 1.2µg from muscle, giving a total intake of 2.2µg/day.

Because of the large difference between this market basket-based exposure of consumers and the smallest study derived NOEL (a factor of 70.000) and based on its previous evaluations of the toxicity of olaquinox, SCAN endorses the JECFA safety evaluations of olaquinox for the consumer and endorses also concentration of 4µg/kg muscle assigned as the MRL.

**Comment** The calculations reveal the expected low level dietary intakes of residues to be free of unacceptable risk for the consumer.

The possibility that the 28 day withdrawal period necessary to achieve low residue levels may not always be observed is sometimes raised as an additional safety concern, despite the fact that this is a time-point in pig production in Europe at which a change in ration composition is customarily made. Because of this possibility, it is comforting to refer to a report which claims that parent carbadox is no longer present in carbadox-containing meat after 20 minutes of cooking (Hassett et al, 1990). It is also noted that the metabolism studies have confirmed the absence of carbadox and desoxycarbadox from edible tissues by 3 days of withdrawal and this has been essentially confirmed in several open literature studies with the exception of one study which reported the detectability of desoxycarbadox up to day 14 of withdrawal.

#### 4.2 THE WORKER (refs 3,4,5,7,8)

Worker safety requires the compliance of the product with the 0.1µg active substance Stauber-Heubach test limit as a means of excluding unacceptable levels of airborne exposure at the workplace. Exposure estimation presumes an 8h shift and a respired volume of 10 cu.m. throughout which an ADI, if available, could be distributed. Although airborne particles up to 50 microns can deposit in the nasal tract, it is only those

smaller than 10 microns which enter and deposit in the lungs. Particles larger than 50 microns are not expected to enter the respiratory tract.

#### 4.2.1 Carbadox

Following discussions with SCAN in the early 80's (ref 8), the product of the original manufacturer of carbadox was tested extensively for worker exposure at several locations in various feed mills as well as on farm. These studies were included in the dossiers submitted to SCAN for its 1982 review. The worst case exposure was claimed to have provided a 1000-fold safety margin. SCAN, in turn, expressed itself satisfied that the potential daily exposure of workers of up to 0.05mg airborne carbadox (presumably incorrectly expressed in the 1982 Opinion as 0.05 mg/kg) per 24h did not threaten its 1978 opinion that the risk to health of workers was negligible.

Also provided in 1982 were studies of QCA in total urines from dust-exposed pigs and workers in pig-feeding houses. These gave negative results with respect to evidence of absorption of carbadox from exposures relevant to feeding and mixings.

The CRAFT GEIE study of unidentified premixes, supplements and feeds across 7 EU States revealed an 85-93% Stauber-Heubach compliance for carbadox-containing samples, (n=54). The balance, except for 1 feed sample, was within a factor of 10 of complying.

In coming to its 1982 conclusion that exposure of workers to airborne carbadox would pose a negligible risk to health SCAN was provided with linear extrapolation risk assessments based both on the "one-hit" and the "multi-hit" models for predicting the "virtually safe dose" (VSD), i.e. the daily dose which carried a life-time increased risk of cancer which was no more than one in a million. The VSDs were  $1.2-4.7 \times 10^{-4}$  mg/kg day for the "one-hit" model and  $2400 \times 10^{-4}$  mg/kg b.wt./day for the "multi-hit" model. These doses were greater than the measured range of feedmill worker exposures ( $< 0.08-0.25 \times 10^{-4}$  mg/kg b.wt./day). The additive formulation in 1982 was shown to comply with the Stauber-Heubach dust release test limit of 0.1µg. This claim was repeated in the data submitted for present review.

#### 4.2.2 Olaquinox

The maximum permitted intake of 2.5µg/kg/day based on the NOEL of 2.5mg/kg/b.wt transforms into a workplace air concentration of 17.5 µg/cu.m., presuming continuous exposure for the entire 8h shift, respiration of all particles and their complete subsequent absorption. In a feed mill study summary, the sponsors showed that actual release was less than 17.5 µg/cu.m

The additive on which data was supplied for this review complies with the mandatory Stauber-Heubach limit of active substance in dust.

The CRAFT GEIE study (Ref. VII) of unidentified premixes, supplements and feeds across 7 EU States revealed an 80-84% Stauber-Heubach compliance for olaquinox-containing samples. Six samples (n=80) remained more than 10x out of compliance.

**Comment** As neither this study nor another (SCAN 97/141) identified the brandname of the products under test, it is impossible to determine the extent to which their findings



apply to the products whose makers supplied data under the “new elements”. The level of non-compliance, which was similar whether applied to premix, supplement or finished feed could be taken to indicate that the formulations tested performed equally well in all three settings even though the Directive 70/524/EEC specification appears to include only the premix formulation.

Both sponsors supplied information which established the compliance of their products with the Stauber-Heubach test limit specified in Directive 70/524/EEC. Added to that is the requirement that exposed workers use dust masks and the knowledge that daily exposure to the additives is not expected in feed mills. The “new elements” provided no additional information on worker exposure to carbadox or olaquinox and SCAN therefore finds no basis for considering its earlier assessments as no longer sound.

## **5. Target Animal Safety (refs 1,4,7,8)**

Concern for target animal safety has arisen because of reports in the open literature which recorded episodes of overdose toxicity and because of studies which reported on the occurrence of adrenal cortex damage as a possible mechanism of that toxicity. While an overdose effect is irrelevant to the proper use of feed additives, it is apparent that a weak effect on the adrenals is possible at user levels.

**Comment** Of importance is, that at approved use levels pigs have superior production indices while receiving the products. That implies that the animals suffer no net physiological let alone a toxicological impact because reduced rather than enhanced production indices would be the expected consequence were that so<sup>11</sup>.

It is also pertinent to note, that the use of carbadox or olaquinox may reduce the prevalence of enteric disease and allows a greater proportional survival of pigs to slaughter weight. In that sense too, it is apparent that the overall influence of the products is beneficial because of decreased pig morbidity and mortality<sup>12</sup>.

Given that the recommended dose should never be exceeded, SCAN recognises the additional risk posed by non-compliance with approved conditions of use of these additives because of doubts over the existence of a safety margin and recommends that the regulations relating to inclusion levels and their monitoring in animal feed are strictly observed.

## **6. Conclusion**

Although the foundation studies on both additives may be considered dated and some would not meet current specifications of design, many were repeated (some several times) and so the compounds benefit from a considerable and in some respects unique body of safety studies. Furthermore, no modern study has differed in conclusion from the earlier ones to an extent which renders the original SCAN Opinions on safety provisions inaccurate.

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<sup>11</sup> Minority opinion of Prof. J. Leibetseder: Cfr. footnote 6.

<sup>12</sup> Minority opinion of Prof. J. Leibetseder: Cfr. footnote 8

The substances share also the property of genotoxicity, differing only in degree. Carbadox is a rodent carcinogen but offers metabolic data which disposes of carcinogenic risk to the consumer provided that its approved conditions of use are observed. Olaquinox is genotoxic, non-carcinogenic but is a rodent tumorigen.

Both sponsored products are presented in well-researched formulations designed to protect the feed operative, but the market contains some products about whose safety performance nothing is known to SCAN. That is a problem which the recent generic products amendment to Directive 70/524/EEC removes. There is little doubt that the possible exposure of workers is a risk, especially from carbadox and it is in this area that the Commission should require increased vigilance at the national level to ensure compliance with the adequate and relatively recent safety measures now in place.

A valuable addition to safety which SCAN urges the Commission to make would be, in recognition of the change in feed mixing practices at the level of the farm, to ensure for the farmer the safety provided by anti-dusting formulations by removing from Directive 70/524/EEC the ambiguity of the anti-dusting provision's applicability to finished feed (ref VII). Also acknowledging the changed circumstances of the feed industry, SCAN recommends that new, extensive studies of real exposures of workers to products of defined origins are now necessary.

When the Commission addresses the question of whether both additives be allowed to remain on the European market, SCAN recommends that within the risk management phase of the risk analysis, due attention be paid to the paucity of the evidence of disease in people additive-exposed as opposed to speculations based on theoretical risks.

The essence of this safety-assessment has been a re-examination of the adverse properties of both additives which had been evaluated previously by SCAN, JECFA and the FDA, and found to be tolerable at expected levels of exposure. For the consumer, there is good security because exposure to carbadox, desoxycarbadox or hydrazine will not occur if user guidelines are followed and, as is also the case for olaquinox, the concentration of total residues to which the consumer will be exposed is, after 28 days of withdrawal, very low and free of toxicological concern. In SCAN's view, it is the potential for exposure at the workplace, and to carbadox in particular, which merited the greater attention. Again, presuming compliance with the anti-dusting specification and the recent restriction of the use of the additives to fully approved feed mills, SCAN was persuaded that security for the operatives was sufficient and that consequently, any risk of adverse health effects from exposure to additive pure substance was negligible.

While recognising

that neither additive has an ideal safety profile in laboratory animal tests,

that there is potential for worker exposure to parent substance

and that there is evidence for a weak effect on adrenal cortex histology in some pigs at user levels in the diet,

SCAN nonetheless concludes,

based on the preceding analysis and discussion of those attributes,

and in the absence of any evidence which supports the occurrence of adverse health effects in consumer, properly protected worker or target animal under approved conditions of use and despite decades of potential exposure to additive concentrations higher than should now be possible under current regulations,

that it is able to maintain its earlier Opinions on the acceptability of the quinoxaline-N-dioxides, carbadox and olaquinox within their previously defined conditions of use<sup>13</sup>.

**SCAN recommends:**

**that the carbadox or olaquinox residue status of pigs slaughtered for consumption at less than 4 months of age be controlled as a matter of urgency,**

**that workplace exposure both to carbadox and olaquinox be re-evaluated,**

**that as a matter of urgency it be supplied with the results of the national monitoring programs of carbadox and olaquinox in animal feeds ,**

**that potential sponsors for these additives under Directive 96/51/EC be informed immediately of the need to commence relevant epidemiological studies of the health status of additive-exposed workers.**

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<sup>13</sup> Minority opinion of Prof. J. Leibetseder: "Carbadox and olaquinox should not be in use furthermore because of reasons mentioned in the footnotes on previous pages."

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2. Commission of the EU: Scientific Conference on the use of growth promoters in meat production. Conclusions of Session I. (Brussels December 1995).
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2. Fifteenth Commission Directive 76/603/EEC of 21 June 1976 amending the Annexes to the Council Directive of 23 November 1970 concerning additives in feedingstuffs (O.J. No. L 198, 23/7/76 p. 10) Action: *Extension of Annex II for further studies. Under part F*
3. Sixteenth Commission Directive 76/933/EEC of 1 December 1976 amending the Annexes to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs. (O.J. No. L364, 31/12/1976 p.18) Action: *Olaquinox enters under part F of the Annex II*
4. 20th Commission Directive 78/58/EEC: of 7 December 1977 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs (O.J. No. L 018, 24/1/78 p. 072) Action: *Extension Carbadox until 31/12/78*
5. Report of the Scientific Committee for Animal Nutrition on the use of Carbadox in feedingstuffs for pigs. Opinion expressed: 6 July 1978. p.7. Second Series (1980) Report 6918 EN. Catalogue N° CDE-NK-80-002-EN-C:
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  20. Commission Directive 86/525/EEC of 27 October 1986 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs (O.J. No. L 310, 5/11/86 p. 19) Action: *Last directive for 1986 concerning 70/524/EEC, therefore the date of 31/12/86 arrived without extension for Carbadox and Olaquinox under the Annex II. In practice this is a banning (principle of a "positive list").*
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  27. Council Directive 87/317/EEC of 16 June 1987 amending Directive 70/524/EEC concerning additives in feedingstuffs in respect of Olaquinox. (O.J. No. L160, 20/6/1987 p.34) Action: Olaquinox in Annex I
  28. Commission Decision 90/38/EEC of 13 December 1989 relating to a proceeding under Article 85 of the EEC Treaty (IV/32.026 - Bayo-n-ox) (Only the German text is authentic). (O.J. No. L021, 26/1/1990 p.71 Action: (A1): *The agreements which were in force from 10 July 1986 to 13 November 1989 between Bayer AG and its customers, under which such customers were required to use 'Bayo-n-ox Premix 10 %' solely to cover their own requirements in their own works, constitute infringements of Article 85 of the EEC Treaty.*(A2): *A fine of ECU 500 000 is imposed on Bayer AG in respect of the infringement referred to in Article 1.*
  29. Commission Directive 90/110/EEC of 19 February 1990 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs. (O.J. No. L67, 15/3/90 p.44) Action: *New definition of Carbadox.*
  30. Report of the Scientific Committee for Animal Nutrition on the possible implication of olaquinox in cases of photoallergic contact-dermatitis developed in pig-farmers. Opinion expressed 18 November 1992. (9th Series, In press).
  31. Commission Decision 96/516/EC of 29 July 1996 on financial aid from the Community for the work of the Laboratoire des médicaments vétérinaires, Fougères, France, a Community reference laboratory for residue testing. (O.J. No. L217, 28/8/1996 p.4) Action: *Analysis of residues of carbadox Olaquinox*
  32. Commission Directive 96/7/EC of 21 February 1996 amending Council Directive 70/524/EEC concerning additives in feedingstuffs). (O.J. No. L51, 01/3/1996 p.45) Action: *New conditions for users (labelling). Follows the advice by SCAN*
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives

85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. (O.J. No. L125, 23/5/1996 p.10. Action: *Residues in meat of carbadox and Olaquinox: (Annex V): Laboratoire de médicaments vétérinaires (CNVEVADO-LMV). La Haute Marche, Javene. F-35135 Fougeres.*

Table 1. Annex I, Section J “Growth Promoters”

EEC No	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum Content mg/kg of feedingstuffs	Maximum Content mg/kg of Complete feedingstuffs	Other provisions
E 850	Carbadox	methyl-3-(2-quinoxalinylnyl methylen) carbazate-n 1 , n 4 - dioxide. Minimum purity 96%. particular features of the authorised preparations - max. carbadox content 5 or 10% - Minimum stability: 24 month - Propionic acid: 0.5% - Soybean oil: 7% - Soybran meal: up to 100%	Piglets	4 month	20	50	Used prohibited at least 4 weeks before slaughter Maximum amount of dust emitted during handling as determined by the Stauber Heibach method <sup>(1)</sup> : 0,1 µg Carbadox Indication on the label of the additives, premixtures and feedingstuffs of safety instructions and warning designed to protect the health of operators and in particular to avoid any exposure to the additive, specially by touch or inhalation

(1) Literature reference: Fresenius Z, Anal. Chem. (1984) 318: 522-524, Springer Verlag 1984.

Table 2: Annex I, under part J “Growth Promoters”

EEC No	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum Content mg/kg of feedingstuffs	Maximum Content mg/kg of Complete feedingstuffs	Other provisions
E 851	Olaquinox	2-(N-2' (hydroxymethyl) carboamoyl)-3-methylquinoxaline N <sup>1</sup> , N <sup>4</sup> - dioxide. Minimum purity 98%. Particular features of the authorised preparations - maximum olaquinox content 10% - Minimum stability: 24 month - Medium: calcium carbonate containing 1,5 of Glyceyl poliethylenglycol ricinoleate	Piglets	4 month	15 50 <sup>(2)</sup>	50 100 <sup>(2)</sup>	Used prohibited at least 4 weeks before slaughter Maximum amount of dust emitted during handling as determined by the Stauber Heibach method <sup>(1)</sup> : 0,1 µg Olaquinox Indication on the label of the additives, premixtures and feedingstuffs of safety instructions and warning designed to protect the health of operators and in



						particular to avoid any exposure to the additive, specially by touch or inhalation with the mention "Warning: Risk of photoallergy for people so predisposed"
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(1) Literature reference: Fresenius Z, Anal. Chem. (1984) 318: 522-524, Springer Verlag 1984.

(2) Milk replacers only