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CVMP assessment report under Article 30(3) of Regulation (EC) No 726/2004

for dapsone as an impurity in veterinary medicinal products containing
sulphamethoxazole or other sulphonamides

Procedure no: EMEA/V/A/075

Assessment report as adopted by CVMP with all information of a confidential nature
deleted



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1 Background information on the procedure

1.1 Request for CVMP opinion

On 22 July 2011, The Netherlands requested the CVMP to draw up an opinion, on the basis of the information currently available, on the implications in terms of animal health and the safety of consumers of the recent finding by the Dutch authorities of dapsone in sulphamethoxazole, which is used as an active ingredient in veterinary medicinal products. Dapsone is a prohibited substance for use in food-producing species. The opinion was requested under Article 30(3) of Regulation 726/2004.

Dapsone was found in feed for fattening pigs on a farm in The Netherlands. The presence of dapsone in the feed appeared to originate from the use of a trimethoprim/sulphamethoxazole premix of which the active ingredient sulphamethoxazole contained dapsone as an impurity.

A preliminary hazard and risk evaluation carried out by the Dutch authorities concluded that dapsone was very likely to be genotoxic. The Netherlands noted that the CVMP had previously assessed dapsone for the purpose of establishing maximum residue limits and concluded that the substance was not genotoxic but their new evaluation took into account more recent data.

In reaching their opinion the CVMP considered the following points as proposed by The Netherlands:

1. Is dapsone to be considered genotoxic?
2. Does sulphamethoxazole from other suppliers than the ones investigated by the The Netherlands contain dapsone? If yes, what are the concentrations?
3. Do the levels of dapsone, present as an impurity in sulphamethoxazole from any supplier, and applied to food producing animals, imply an unacceptable risk for consumers?
4. Do the levels of dapsone, present as an impurity in sulphamethoxazole from any supplier, imply an unacceptable risk for animals?
5. Could the finding of dapsone as impurity in sulphamethoxazole be relevant for other sulphonamides?
6. Does the CVMP consider it necessary to take risk management measures? If yes, what risk management measures and communication does the CVMP consider appropriate?

1.2 Steps taken during the procedure

The procedure under Article 30(3) started on 15 September 2011.

The CVMP adopted an opinion at their July 2012 meeting.

2 Scientific discussion

2.1 Introduction

Dapsone (4, 4'-diamino-diphenyl sulfone) is an antibacterial that acts in the same way as sulphonamides by inhibiting the dihydrofolic acid synthesis via competition with para-aminobenzoic acid for the active site of dihydropteroate synthetase. Dapsone also has anti-inflammatory properties.

Dapsone is used in human medicine in combination therapy with rifampicin and clofazimine for the treatment of leprosy. It is also used to treat (prevent) *Pneumocystis pneumonia* in HIV patients. The substance is used in combination with pyrimethamine in malaria prophylaxis. Dapsone is used orally to

treat severe acne and topically to treat other skin diseases such as dermatitis herpetiformis and mild to moderate acne as topical medication.

In veterinary medicine dapsone had been used either alone or in combination with other chemotherapeutic agents for the intramammary treatment of bovine mastitis, for the oral treatment of bovine coccidiosis and the intra-uterine treatment of endometritis.

The use of dapsone as an active ingredient in veterinary medicinal products for food producing animals has been prohibited since January 1994 following the conclusion of its evaluation for the establishment of maximum residue limits (MRLs). Initially a provisional ADI of 3.5 µg/kg was established and provisional maximum residue limits of 25 µg/kg were set in muscle, liver, kidney, fat and milk for all food producing species. Further information on teratogenic and reproductive effects was requested at that time. As no data were provided before the expiry date of the provisional MRL (1 January 2004), and the safety concerns identified, the compound was entered into Annex IV of Regulation (EEC) No 2377/90 and is now listed in table 2 (prohibited substances) of the annex to Regulation (EU) No 37/2010. Its use as pharmacologically active substance for food producing species is therefore not allowed in the EU.

Dapsone was recently found as a contaminant in trimethoprim/sulphamethoxazole-containing veterinary medicinal products used to treat fattening pigs on a farm in The Netherlands. The active ingredient sulphamethoxazole was contained dapsone as an impurity linked to the synthesis process. The exact reason for the presence of dapsone as contaminant in sulphamethoxazole is currently unclear.

The quality standard of sulphamethoxazole is described in a European Pharmacopeia (Ph. Eur.) monograph. The Ph. Eur. monograph for sulphamethoxazole mentions limits for six impurities, however, dapsone is not one of the listed impurities. According to the Ph. Eur. monograph other impurities may be present up to a level of 0.1%. The extent of dapsone contamination was found to be well below the limit of 0.1% for any other impurity, as allowed in the Ph. Eur. sulphamethoxazole monograph. However, if the unknown impurity in a drug substance is considered to be a genotoxic substance, the maximum limit of 0.1 % valid for 'ordinary' impurities does not apply. This is established in the guideline on the limits of genotoxic impurities for human medicines (EMA/CHMP/QWP/251344/2006).

While the combination trimethoprim/sulphamethoxazole or co-trimoxazole is widely used in human medicine for a variety of bacterial infections, its use in veterinary medicine is more limited. In veterinary medicine trimethoprim is often combined with sulfadiazine or sulfadoxine. Sulphamethoxazole alone is not used in veterinary medicines.

A preliminary analysis of the Dutch Food and Consumer Product Safety Authority came to the conclusion that dapsone was very likely to be genotoxic. Based on the assumption that dapsone is genotoxic and taking into account the levels of dapsone found in the veterinary medicinal products a risk assessment was performed by the Dutch authorities which took into consideration the CHMP guideline on the limits of genotoxic impurities (CPMP/SWP/5199/02, EMA/CHMP/QWP/251344/2006). This guideline uses a threshold of toxicological concern (TTC) approach to establish a maximum exposure limit that can be accepted for genotoxic impurities. The value of 1.5 µg per person per day is identified as the maximum acceptable dose of a genotoxic impurity for a patient (for very potent carcinogenic compounds a more conservative TTC may be used). Exposure at this level is considered to present an acceptable increased risk when considering the benefit the patient receives by taking the medicine. In the hazard analysis performed by the Dutch authorities the TTC value was lowered by a factor of 10 to take account of the fact that there is no benefit to the consumer from the ingestion of food coming from animals treated with sulphamethoxazole contaminated with dapsone.

The outcome of the risk assessment by the Dutch authorities can be summarised as follows:

- It is assumed that dapsone is very likely genotoxic, presumably through a clastogenic mechanism. Nevertheless exposure extrapolations allow to conclude that the consumer risk is negligible in case of poultry and cattle meat. There is however a risk for the consumer in case of meat from pigs, and measures are required to reduce this risk. The Dutch authorities propose to apply a longer withdrawal period.
- The animal health risk for poultry, calves and pigs is negligible taking into account their short life span, but for dairy cows it was not possible to completely exclude an animal health risk.

Subsequently The Netherlands requested the CVMP for clarification of six questions as outlined above.

2.2 CVMP assessment of dapsone contamination in veterinary medicinal products containing sulfamethoxazole

2.2.1 Is dapsone to be considered genotoxic?

From a historical point of view, dapsone has previously been scientifically assessed (1990-1993) by the CVMP for the establishment of maximum residue limits according to the requirements of Council Regulation (EEC) 2377/90 (repealed by Regulation (EC) No 470/2009). The substance is included in table 2 (Prohibited substances) of the annex to Regulation (EC) No 37/2010) due to insufficient data concerning reproductive toxicity and teratogenicity. The initial MRL summary report (October 1990), indicates that mutagenicity testing of dapsone, its N-acetylated and hydroxylated metabolites in bacterial and mammalian cells with and without metabolic activation indicated negative results. It was also noted in the MRL summary report that carcinogenicity testing of dapsone in rats, at high doses, showed evidence of an increased incidence of tumours in the spleen and peritoneum of males and of thyroid tumours in both sexes. Studies with mice gave no evidence for carcinogenicity. Epidemiological data on carcinogenicity of dapsone in humans appeared to be lacking. Based on the results of short-term mutagenicity testing dapsone was considered as a carcinogen with a threshold based mechanism of action.

Assessment by the Dutch Food and Consumer Product Safety Authority report

The following studies were made available as part of the report provided by The Netherlands.

| Test | Result | Reference |
|---|---------------|-------------------------------------|
| Ames (TA98, TA100) +/- S9 mix | - | Peters et al, 1983 |
| Ames (TA98, TA100) +/- S9 mix | - | Tanaka et al, 1985 |
| Ames (TA98, TA100, TA1535, TA1537) +/- S9 mix | - | NTP (Ref. Dunkel et al, 1985) |
| <i>In vitro</i> mouse lymphoma test | - | NTP (Ref. Mc Gregor et al, 1988) |
| <i>In vitro</i> sister chromatid exchange test (2 studies) | - | NTP, 1977 |
| <i>In vivo</i> mouse (bone marrow, spermatocytes) -Micronucleus test -Chromosomal aberrations | + | Roy and Das, 1988 |
| <i>In vitro</i> human leukocytes -Chromosomal aberrations | (+) | Beiguelman et al, 1975 |
| <i>In vitro</i> chromosomal aberrations (2 studies) | + | NTP, 1977 |
| Humane skin fibroblasts (leprosy patients) -Chromosomal aberration | + | Hackel and Beiguelman, 1985 |

| | | |
|---|---|------------------------|
| Human peripheral blood lymphocytes (leprosy patients) -Chromosomal aberrations -Micronucleus test -Comet assay | + | Kalaiselvi et al, 2002 |
| Human peripheral blood lymphocytes (leprosy patients) -DNA damage | + | Gandhi and Singh, 2004 |

In the Dutch report, dapsone was considered as having a clastogenic potential *in vitro*, based on a significant increase of frequencies of structural aberrations in 2 studies, however only seen at the highest concentrations. Due to missing data on cytotoxicity it was considered not possible to decide whether dapsone was a real clastogen or just a "high toxic genotoxin". A principal *in vivo* study indicated a dose-related increase in bone marrow cells and in spermatocytes with chromosomal aberrations in mice which supported the possible genotoxic nature of dapsone, probably through a clastogenic mechanism. In addition, further studies showed tumours in multiple sites in rats (spleen, peritoneum and thyroid). A peer reviewer of the Dutch Food and Consumer Product Authority also concluded that dapsone was very likely to be genotoxic, presumably through a clastogenic mechanism.

CVMP assessment taking into account the assessment provided by The Netherlands

In *in vitro* conditions, the AMES tests (Peters et al, 1983 ; Tanaka et al, 1985 et NTP Dunkel et al, 1985), the mouse lymphoma tests (Gregor et al, 1988) and the sister chromatid exchange test (2 studies, NTP 1977) showed clearly negative results which can lead to the conclusion that *in vitro* studies with dapsone have yielded negative results. Nevertheless, the results of *in vitro* chromosomal aberrations tests (2 studies, NTP, 1977) showed positive effects. Even if these studies were not performed according to current standard guidelines and therefore their results should be treated with caution, the CVMP considered the results useful in the current assessment of genotoxicity of dapsone.

In vivo conditions, only one study was available (Roy and Das, 1988) in mouse using different tests (micronucleus test and chromosomal aberrations test). The oral dosing regimen of the *in vivo* study was not according to the current testing guidelines, but the measurements of DNA damage were. Nevertheless, it was correctly noticed that this study was performed in line with recently proposed guidance on genotoxicity testing of pharmaceuticals (ICH, 2010) and chemicals (ECHA, 2008b). Therefore, the results of this study are considered valid. The results show that dapsone induced structural chromosomal aberrations in the bone marrow, which were statistically significantly different from controls at all tested doses (including and excluding gaps) and treatment periods. Also a statistically significant increase in micronuclei at all tested doses compared to controls and a significant higher incidences of chromosome breaks in spermatocytes were observed for the highest dose (80 mg/kg bw/day for 4 weeks) and longest treatment time (40 mg/kg bw/day and 8 weeks).

Several tests using human cells (from leprosy patients) were also presented. The results obtained should be taken with caution since cells were taken from leprosy patients treated with a combination of products and that leprosy itself may generate DNA-damage (Gandhi and Singh, 2004). Nevertheless, these results should not be totally excluded since a correlation was seen between the therapy-induced DNA damage and the administered dose. The results reveal that patients undergoing therapy had significantly greater DNA damage as compared with untreated patients, indicating that bacterial infection and drug therapy are likely to be causal factors. Data from studies of leprosy patients, although not directly attributed to dapsone treatment alone, support the assumption that the compound may have a damaging effect on DNA.

On the basis of the available data reviewed by the Dutch report, the CVMP considered that dapsone was likely to be genotoxic *in vivo* in somatic and in germ cells, presumably through a clastogenic

mechanism. It was agreed however to request further expert advice from an Ad hoc Expert Group (AHEG). The questions addressed to the AHEG were the following:

- 1) Does the expert group consider the data set provided as a sufficient basis for assessing the genotoxic potential of dapsone?
- 2) Does the expert group support the conclusions on genotoxicity as formulated in the assessment report from the rapporteur and co-rapporteur?
- 3) Does the expert group consider dapsone as a genotoxic carcinogen?

The AHEG on dapsone met on 5 December 2011.

The AHEG agreed that positive results were obtained in some *in vitro* and *in vivo* genotoxicity studies and concluded that dapsone is genotoxic in mice. The AHEG also agreed that negative results were obtained in mice carcinogenicity assays and positive results were obtained in rat carcinogenicity studies. However, AHEG did not reach consensus on the interpretation of the mechanism of action of dapsone that leads to these results. Some experts of the group considered that, taking into account the absence of carcinogenicity in mice, the *in vivo* mutagenicity observed in mice has no consequence in terms of carcinogenicity. They postulated that the *in vivo* mutagenicity observed in mice is the consequence of folate deficiency and that positive results observed in *in vitro* chromosomal aberration studies (NTP) should be viewed as the consequence of cytotoxicity. It was considered that the pharmacological mode of action of dapsone may be inhibition of dihydrofolic acid synthesis via competition with para-aminobenzoic acid for the active site of dihydropteroate synthase. When given orally to mice this compound will affect gut flora with an effect to diminish folate availability and absorption for the animal. Consequently, folate deficiency would be induced. This would result in impaired DNA synthesis and subsequently chromosome breakage in susceptible cells. For other experts of the group, this hypothesis could not be accepted as such taking into account the fact that positive results were observed in the carcinogenicity studies in rats and that positive results were also observed in cells from leprosy patients treated by dapsone (even if these results should be read with caution). They considered that further data would be necessary to confirm that hypothesis on the mode of action in terms of carcinogenicity.

The CVMP noted the differences in the interpretation of the results of the *in vitro* NTP chromosomal aberration studies. The CVMP considered that, based upon the *in vivo* data (1988), dapsone should be considered genotoxic as a conservative approach and that the hypothesis made by some members of the AHEG concerning the genotoxic mechanism would need to be confirmed by further experimental data.

The Netherlands agreed to perform a new *in vivo* study in mice using the intraperitoneal route of administration to confirm the hypothesis of folate deficiency being the probable mechanism for the observed carcinogenicity (NOTOX Project 499073, Verban, 2012). In such a study no folate deficiency would appear in the gut flora microorganisms following exposure through intraperitoneal route and this should lead to negative results if the hypothesis was correct.

The CVMP noted the EFSA on dapsone concluding that the substance is not genotoxic *in vitro* and *in vivo* (The EFSA Journal (2005) 248, 1-16). The EFSA opinion was in part based on the result of a mammalian erythrocytes micronucleus assay where the results indicated that, a single intraperitoneal administration of dapsone at dose up to 100 mg/kg did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female ICR mice. Therefore, it was concluded that dapsone was negative in the mouse micronucleus assay. This study was also made available to CVMP.

In the meantime the Dutch authorities had already initiated the new study using also intraperitoneal administration in mice. The additional Dutch study was performed in order to obtain information on the

clastogenicity and aneugenicity of dapsons by measuring the increase in the number of micronucleated polychromatic erythrocytes in mouse bone marrow. The study procedures were based on the ICH Topic S2A, Topic S2B and S2(R1) and were in accordance to the OECD guideline n°474 (Mammalian erythrocyte micronucleus test). In the main micronucleus study, 5 male mice were treated per sampling time in each treated group. Negative and positive controls were correctly used and the dose levels as well as the sampling times were correctly chosen according to the cytotoxicity observed in the range finding study.

The animals of the group which were treated with 100 mg dapsons/kg bw showed a decrease in the ratio of polychromatic to normochromatic erythrocytes compared to the vehicle controls, demonstrating toxic effects on erythropoiesis in 3 animals which shows evidence that the test substance or a reactive metabolite, reached the target tissue (bone marrow).

The AHEG was consulted on the results of the study and concluded that the micronucleus test using a parenteral route of administration was negative. The negative results in this test were considered consistent with the hypothesis that the positive result in the oral micronucleus test was due to clastogenicity caused indirectly as a result of the oral administration of dapsons, causing folate deficiency in mice by affecting metabolism of the gut flora by the mechanism. The AHEG considered that it was reasonable to conclude that dapsons does not have a direct genotoxic effect and that the clastogenicity and carcinogenicity seen in laboratory animals after long-term oral exposure to high doses of dapsons might be the consequence of a non-genotoxic mode of action.

The CVMP endorsed the conclusions of the AHEG.

This conclusion is also in line with the EFSA opinion which concludes that dapsons is not genotoxic *in vitro* and *in vivo* (The EFSA Journal (2005) 248, 1-16).

As dapsons is not considered to have a direct genotoxic effect it follows that the observed carcinogenicity was the result of an indirect effect and that an exposure threshold exists, below which carcinogenicity will not occur. The threshold can be estimated based on the NOEL/LOEL seen in the most relevant study, and a margin of exposure (or margin of safety) can be calculated in relation to the estimated threshold.

2.2.2 Does sulfamethoxazole from other suppliers than the ones investigated by the Dutch authorities contain dapsons? If yes, what are the concentrations?

On request from EMA, information was received from Member States (MSs) on suppliers of sulphamethoxazole for their authorised products for food-producing animals. Not all Member States provided the requested data.

There are currently three active substance suppliers known to the EMA who manufacture sulphamethoxazole for use in veterinary medicinal products on the market in the EU/EEA. All of them are listed as Certificate of Suitability (CEP) holders by the EDQM:

| | |
|--|------------------------|
| Shouguang Fukang Pharmaceutical Co Ltd: | R0-CEP 2007-332-Rev 00 |
| Virchow Laboratories Limited: | R1-CEP 1999-172-Rev 01 |
| Southwest Synthetic Pharmaceutical Co. Ltd.: | R1-CEP 1999-097-Rev 01 |

The Ph. Eur. monograph of sulphamethoxazole mentions limits for six specific impurities (A-F), but dapsons is not listed. According to this monograph other individual impurities may be present up to a level of 0.1 % (any other impurity). Whilst little information is known about the actual levels of impurities in sulphamethoxazole, according to the assessment reports for the CEPs, provided by the EDQM the specifications of all sulphamethoxazole suppliers are in line with the Ph.Eur. monograph.

In addition analyses by the Dutch Institute of Food Safety (RIKILT) confirmed that sulphamethoxazole from two active substance manufacturers, who supply their active substance to veterinary medicines producers in the Netherlands, meet the Ph.Eur. monograph requirements. The extent of dapsone contamination in the drug substance was below the limit of 0.1 % for any other impurity, as fixed in the Ph. Eur. sulphamethoxazole monograph. The levels of dapsone found in samples of sulfamethoxazole ranged from 8.4 to 59 µg/g.

With regard to the third sulphamethoxazole manufacturer, results of the analyses performed by the RIKILT were not available yet. Therefore it cannot be excluded that this manufacturer also produces sulphamethoxazole with a certain level of dapsone contamination.

According to the information on the different routes of synthesis defined in assessment reports for the CEPs provided by the EDQM, in principle all three syntheses follow the scheme as outlined in the commentary to the Ph. Eur. (Kommentar zum Europäischen Arzneibuch, Band 8, Sulfamethoxazol, 5.0/0108, 2006) for sulfamethoxazole.

The intermediate 5-methylisoxazol-3-amine and the possible reagents to build this substance can be excluded as source of dapsone for structural reasons. A possible source could be the second main intermediate 4-acetaminobenzenesulfonyl chloride. The influence of the different synthetic routes on the extent of dapsone formation cannot be currently estimated. The formation of dapsone later in the synthesis is also possible. Therefore a correlation between the specific synthetic route of a certain manufacturer and the amount of dapsone formation cannot be evaluated without further information from the active substance manufacturer and/or a more in depth investigation.

2.2.3 Do the levels of dapsone present as an impurity in sulphamethoxazole from any supplier, and applied to food producing animals, imply an acceptable risk for consumers?

The Dutch Food and Consumer Product Safety Authority (Ministry of Economic Affairs, Agriculture and Innovation) prepared a consumer risk assessment of dapsone contamination in veterinary medicinal products containing sulfamethoxazole/trimethoprim.

The report contains an exposure assessment of certain products for poultry (broilers), pigs and cattle. The assessment is based on data on dapsone contents from a chemical analysis of veterinary medicinal formulations. The highest dapsone concentrations were 5.2 mg/kg in oral solutions (broiler/pigs) and 2.9 mg/kg in oral powders (pigs). In products for pigs and cattle (injectable liquids) the content was estimated with 6.2 mg/l. The daily doses of dapsone for the animals were estimated with 1.72 µg/kg bw for broilers (for 3 to 4 days), 1.45 µg/kg bw in pigs (over 4 to 7 days) and 0.31 µg/kg bw in cattle (5 consecutive days).

The assessment was based on these values and pharmacokinetic modelling to derive a (mean) steady state concentration for dapsone in meat (muscle) and plasma. Maximum levels of dapsone in broiler, pig and beef meat were estimated as 3.1 µg/kg, 5.2 µg/kg and 1.29 µg/kg, respectively. Based on these data approximate elimination periods were extrapolated using elimination half-lives in plasma as surrogate parameter. As toxicological reference point for these estimates a TTC value of 0.15 µg/person/day was used (0.3 µg/kg muscle if a standard food basket of 500 g meat is used). These elimination periods were compared to existing withdrawal periods for the sulphamethoxazole/trimethoprim products in The Netherlands. The results suggested that if existing withdrawal periods are observed the risk to ingest residues in meat higher than the TTC is negligible for broilers and cattle but not for pigs. The assessment does not include quantitative exposure estimates for milk, but for the purpose of this assessment, it may reasonably be assumed that residues

in commercial milk are highly diluted with uncontaminated milk and would not constitute a risk to the consumer.

According to the Dutch report maximum levels of dapsons in broiler, pig and beef meat were assumed (theoretical considerations) to be 3.1 µg/kg, 5.2 µg/kg and 1.29 µg/kg, respectively. Based on a rudimentary calculation an ingestion of 500 g meat per day would mean that the consumer is in a worst case scenario exposed to 2.6 µg dapsons per day or 0.0433 µg/kg bw per day (bodyweight: 60 kg).

Based on the findings that dapsons is considered a non-genotoxic carcinogen, it was considered adequate to make safety span calculations using a threshold approach based on the most relevant available safety study.

The most relevant study in relation to the raised concern is a positive (worst case) rodent carcinogenicity study i.e. rat NTP carcinogenicity study (1977):

- administration of dapsons in feed at doses of 600 and 1200 ppm for 78 weeks;
- corresponding to daily doses of 42 and 84 mg/kg (standard assumption for calculation: rat food consumption: 30 g/day; rat bodyweight: 0,425 kg);
- At 104-106 weeks significant tumour incidence (mesenchymal tumours of the spleen & peritoneum) at both dose levels (13/35 low-dose males and 22/33 high-dose males)
- No observed effect level (NOEL) can be established; Lowest observed effect level (LOEL): 42 mg/kg.

Based on the assumption that dapsons is not a genotoxic carcinogen it is possible to calculate a margin of safety (MOS).

MOS: $\frac{\text{LOEL}}{\text{Exposure}}$

MOS: $\frac{42000 \text{ µg/kg}}{0.0433 \text{ µg/kg}} = 970000$

The CVMP concludes that, even taking into account that only a LOEL and not a NOEL could be determined, a margin of safety of 9.7x10⁵ is, in the absence of a direct genotoxic potential, sufficient and comfortable enough to conclude that the carcinogenic risk for the consumer from sulfamethoxazole residues contaminated with dapsons is negligible.

It should also be noted that these products have withdrawal periods that add to the safety of the product next to the safety margin.

The assessment does not include quantitative exposure estimates for milk, but for the purpose of this assessment, it may reasonably be assumed that residues in commercial milk are highly diluted with uncontaminated milk and would not constitute a consumer risk.

In addition to calculating a margin of safety and using the same LOEL, the CVMP also calculated the maximum daily exposure of dapsons that could be considered safe in terms of risk of cancer:

Maximum daily exposure considered safe in terms of cancer risk (mg/kg):

$$= \frac{\text{NOEL or LOEL (mg/kg)} \times \text{Weight adjustment (60 kg)}}{\text{F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5 (Modifying factors)}}$$

The LOEL of 42 mg/kg identified in the positive (worst case) rodent carcinogenicity study i.e. rat NTP carcinogenicity study (1977) described above under 2.2.3 and the following uncertainty factors were considered:

F1 = 10 to account for extrapolation from rats to humans

F2 = 10 to account for differences between individual humans

F3 = 1 because of long duration of treatment (78 weeks)

F4 = 10 because an oncogenic (serious) effect was reported

F5 = 10 because a NOEL was not established (tumour incidence increased at both dose levels)

Resulting in the following calculation:

$$= \frac{42 \text{ mg/kg} \times 60 \text{ kg}}{10 \times 10 \times 1 \times 10 \times 10} = 0,252 \text{ mg/person day or } 4.2 \text{ } \mu\text{g/kg bw}$$

Both the margin of safety and the maximum daily exposure considered safe in terms of cancer risk (assuming dapson impurity of 60 µg/g) suggest that the worst case consumer exposure (estimated to be 0.0433 µg/kg bw per day) does not present a risk for the consumer.

It is important to note that the calculation of a margin of safety and the calculation of a maximum daily exposure relates specifically and only to the genotoxic and carcinogenic properties of dapson and are distinct from an ADI that would require data on additional endpoints .

2.2.4 Do the levels of dapson, present as an impurity in sulphamethoxazole from any supplier imply an unacceptable risk for animals?

In the advice about the risks of a veterinary medicine contaminated with dapson from the Office for Risk Assessment and Research (BuRO), the following risk assessment for animal health was presented: "Due to the short time that broilers, fattening pigs and calves live before they are slaughtered it does not appear to be likely that the low dosages of dapson form a risk to animal health. For dairy cows, which live longer, such a statement may not be defensible and it may not be possible to exclude a risk to animal health, such as the development of tumours. However, no information is available on this topic."

The CVMP agree with the risk assessment of the Dutch authorities in so far that animal health risks from sulphamethoxazole contaminated with dapson are considered to be negligible for broilers, fattening pigs and calves.

When evaluating cancer risks for dairy cows the following should be taken into account:

Cattle are treated with injectable liquids, the maximum content of dapson in these products was estimated to be 6.2 mg/l. A rough calculation assuming a dairy cow bodyweight of 600 kg treated with 0.1 ml per kg and day would result in application of 372µg dapson per animal and per day or 0.62 µg/kg/day.

Provided the same assumptions for human and animal health risk the following MOS for dairy cows can be calculated:

$$\text{MOS: } \frac{42000 \text{ } \mu\text{g/kg}}{0.62 \text{ } \mu\text{g/kg}} = \underline{67742}$$

A margin of safety of 6.77×10^4 is considered a sufficient high margin of safety in dairy cattle and therefore animal health risks from sulfamethoxazole contaminated with dapsone are considered to be negligible also for dairy cattle.

2.2.5 Could the finding of dapsone as impurity in sulphamethoxazole be relevant for other sulphonamides?

It is currently not clear at what stage during the synthesis of sulphamethoxazole the impurity dapsone is formed. The most probable hypothesis is that 4-acetaminobenzenesulfonyl chloride is the key compound to the understanding of the occurrence of dapsone: either dapsone is formed in the course of 4-acetaminobenzenesulfonyl chloride synthesis or dapsone is built up in a later stage of synthesis of sulphamethoxazole where 4-acetaminobenzenesulfonyl chloride could react with acetanilide to give a diacetylated dapsone derivative as a by-product which later is hydrolysed under acidic conditions to give dapsone.

If 4-acetaminobenzenesulfonyl chloride would really be identified as the compound from which dapsone formation takes place or which contains dapsone as impurity from its own synthesis, this would lead to implications for other sulfonamides which are also synthesised using 4-acetaminobenzenesulfonyl chloride as starting material (intermediate), e.g. sulfanilamide, sulfadiazine, sulfadimidine, sulfadoxine. For sulfanilamide it is known that the impurity 4,4'-diacetylaminodiphenyl sulfone can occur (commentary to the Ph. Eur. (Kommentar zum Europäischen Arzneibuch, Band 8, Sulfanilamide, 5.0/1571, 2006)), which can easily be converted to dapsone (4,4'-diaminodiphenyl sulfone). The monograph on sulfanilamide does not list impurities.

There is another sulfonamide described in the Ph. Eur., sulfacetamide sodium, for which the transparency list of the monograph mentions dapsone as impurity (impurity D). Concerning the dapsone limit the monograph refers to the general acceptance criterion for other/unspecified impurities (0.10 %) and/or to the general monograph 'Substances for pharmaceutical use' (2034). In the synthesis of sulfacetamide sodium the structurally closely related compound 4-aminobenzenesulfonyl chloride is used instead of 4-acetaminobenzenesulfonyl chloride. However, sulfacetamide sodium is not used in veterinary medicines.

Assuming that 4-acetaminobenzenesulfonyl chloride is the key substance in most syntheses of sulphonamides, it means that other sulfonamides used to treat food producing animals could theoretically be contaminated by dapsone. According to the information provided by the Member States, the following sulfonamides are used in veterinary medicinal products to treat food producing animals in Europe:

Sulfanilamide, sulfadiazine, sulfadimidine, sulfadoxine, sulfathiazole, sulfamethiazole, sulfamerazine, sulfadimethoxine, sulfamethoxypyridazine, sulfaguandin, sulfaclozine (= sulfachlorpyrazine), sulfachlorpyridazine, sulfaquinoxaline, sulfapyridine. Some of these compounds are used as the respective sodium salts.

Other sulfonamides not included in that list but used in veterinary medicines are sulfasalazine and sulfalen (both used also in human medicines) and sulfaphenazole.

The conclusion can be drawn that most other sulfonamides used to treat food producing animals will be probably contaminated by dapsone. Currently no concrete data are available; therefore it is not possible to give figures. The situation might also be different from one sulphonamide to another and does depend very much on the synthesis of the compound in question.

As a rough estimate one could assume that the levels of dapsons that might be present are in the same order of magnitude in other sulfonamides. Therefore with regard to consumer and animal safety considerations, the (theoretical) risks can be assumed and calculated in the same manner as discussed under points 2.2.4 and 2.2.5. for sulfamethoxazole contaminated by dapsons.

In conclusion the risk for humans in terms of carcinogenicity is likely to be negligible when consuming meat from pigs, cattle or chickens treated with VMPs containing other sulfonamides contaminated with dapsons.

2.2.6 Is it necessary to take risk management measures? If yes, what risk management measures and communication does the CVMP consider appropriate?

The CVMP concludes after re-evaluation of all available data from mutagenicity studies including data from the EFSA and the recently performed *in vivo* MN-test (Verban, 2012), and consultation of the AHEG, that:

- Dapsons is not considered to be a genotoxic substance.
- Carcinogenicity and clastogenicity seen in laboratory animals after long-term oral exposure to high doses of dapsons are most probably the consequence of a non-genotoxic mode of action. Therefore a threshold-related approach was used to calculate the margin of safety.
- For consumers, the carcinogenic risk from residues from animals that have been treated with oral or injectable products containing sulfamethoxazole contaminated with dapsons is considered to be negligible.

The CVMP considers that no risk management measures are required with regard to sulfamethoxazole currently used as active ingredient in veterinary medicinal products because the overall safety assessment has revealed that dapsons when found at impurity levels up to 60 µg/g sulfamethoxazole is neither of consumer safety nor of animal safety concern.

Sulfonamides are used in medicinal products for human use. Considering that the presence of dapsons in sulfamethoxazole and likely in other sulfonamides results from the synthesis process it is likely that dapsons is also present in sulfonamides used in human medicines. The CVMP therefore recommends that the relevant elements of this evaluation are brought to the attention of the CHMP for information and further consideration, if considered appropriate.

In addition, the CVMP recommends that the Agency liaises with the EDQM for consideration of the relevant parts of the assessment in the context of the monographs in the Ph. Eur. for sulfamethoxazole and sulfonamides in general. In particular the EDQM may wish to consider revising the monograph to include a specific limit for dapsons and a corresponding test method for its quantification.

Taking into account that dapsons is classified as a prohibited substance for use in food producing species under the MRL legislation (table 2 of the Annex to Regulation 37/2010) consideration could be given to establishing a reference point for action for the substance, by the European Commission. Should EFSA be requested by the European Commission to perform a risk assessment in this context (the work done to derive a maximum daily exposure considered safe in terms of cancer risk) could be relevant. The CVMP therefore recommends that the Agency submits this assessment to the European Commission and EFSA for information.

3 Overall conclusions

Based on the overall available data, the CVMP is of the opinion that dapsone is not considered to be a genotoxic substance. Carcinogenicity seen in laboratory animals after long-term oral exposure to high doses of dapsone is most probably the consequence of a non-genotoxic mode of action.

Further to a request to Member States, the Agency received information that there are currently three active substance suppliers that manufacture sulfamethoxazole for use in veterinary medicinal products on the market in the EU/EEA, all listed as Certificate of Suitability (CEP) holders by the EDQM.

The data available on the concentration levels of dapsone in sulphamethoxazole are the data provided by The Netherlands which concerned sulphamethoxazole supplied by Virchow and Shouguang. These data confirmed that sulphamethoxazole used in veterinary medicines in The Netherlands meets the Ph.Eur. monograph requirements i.e. is below 0.1 %.

Taking into account that dapsone is considered a non-genotoxic carcinogen and that the substance was found as an impurity within the limit of 0.1%, the AHEG considered it not necessary to require further evidence to demonstrate an absence of carcinogenic risk from the concentrations of dapsone to which humans will be exposed when eating meat from animals that have been fed with feed mixed with a premix containing sulfamethoxazole that was found contaminated with dapsone.

In addition, pharmacokinetic modelling based on the exposure situation for the specific products used in The Netherlands and based on the lowest observed effect level (LOEL) from the most relevant safety study, which is the positive rodent carcinogenicity study, allowed the estimation of a margin of safety (MOS) of 9.7×10^5 . Using the same LOEL and additional safety factors, a maximum daily exposure to be considered safe in terms of cancer risk to the consumer was calculated to be 0.252 mg/person day or 4.2 µg/kg bw which is considerably higher than the worst case exposure of 0.0433 µg/kg assuming dapsone impurity of 60 µg/g.

In these calculations withdrawal period considerations (dapsone levels decrease in these periods) were not taken into account and therefore additionally attribute to the conservative risk calculation approach.

The CVMP concludes that, even taking into account that only a LOEL and not a NOEL could be determined, a margin of safety estimated and the maximum daily exposure in terms of cancer risk constitute a sufficient and comfortable enough margin to conclude that the carcinogenic risk for the consumer from sulfamethoxazole residues contaminated with dapsone is negligible.

With regard to the animal health risk, it is concluded that there is no risk for chickens and pigs, due to the short time that these animals live before they are slaughtered. The margin of safety calculation carried out for cattle (dairy cows) revealed also that the risk for dairy cows in terms of carcinogenicity is negligible, when receiving veterinary medicinal products containing sulphamethoxazole products contaminated with dapsone.

It is currently not clear at what stage during the synthesis of sulphamethoxazole the impurity dapsone is formed. The most probable hypothesis is that 4-acetaminobenzenesulfonyl chloride is the key compound to the understanding of the occurrence of dapsone. 4-Acetaminobenzenesulfonyl chloride is the key substance in most syntheses of sulfonamides. The conclusion can be drawn that also most other sulfonamides used to treat food producing animals will probably be contaminated by dapsone. Currently no concrete data are available, and this issue would have to be further investigated. The situation might also be different from one sulphonamide to another and depends very much on the synthesis of the compound in question.

The risk for humans in terms of carcinogenicity is likely to be negligible when consuming meat from pigs, cattle or chickens treated with veterinary medicinal products containing other sulfonamides contaminated with dapsone.