OPINION ON SPECIFIC QUESTIONS FROM THE COMMISSION
CONCERNING THE EVALUATION OF INDOXACARB IN THE CONTEXT OF
COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants on 18 July 2002)
A. Title

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF INDOXACARB

(Opinion expressed by the Scientific Committee on Plants, 18 July 2002)

Terms of Reference

The Scientific Committee on Plants (SCP) is requested to respond to the following question in the context of the Commission’s work on the implementation of Council Directive 91/414/EEC concerning the placing of plant protection products on the market:

1. Can the Committee comment on the NOEL\(^1\) for effects on red blood cells in rats?

2. The Committee is requested to comment on the adequate basis for the derivation of an Acute Reference Dose for indoxacarb.

C. Opinion of the Committee

Opinion on question 1

Changes in some RBC\(^2\) parameters (RBC count, hematocrit, haemoglobin level) after repeated indoxacarb treatment were generally slight (<10% and within the reference values, although sometimes significantly lower than control values) and not accompanied by a significant reticulocytosis. The observed haemosiderin deposits in spleen and liver, and spleen and bone marrow hyperplastic response should be considered secondary physiological response to the increased RBC turnover. The overall picture is that of a mild haemolytic effect, possibly due to oxidative stress on RBCs, with a very shallow dose response curve. While the Committee concluded that a clear NOEL could not be established, the absence of reticulocytosis and the slight changes (<10%) in treated animals with values mostly within the range of normal values lead the Committee to consider that these effects are non adverse up to 40 ppm.

Opinion on question 2

The Committee is of the opinion that the general and non-specific signs of toxicity observed in the acute neurotoxicity study in rats (NOAEL\(^3\) 12.5 mg/kg) can be used as a basis for the derivation of an ARfD.

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\(^1\) No Observed Effect Level  
\(^2\) Red Blood Cells  
\(^3\) No Observed Adverse Effect Level
A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF INDOXACARB.

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C. BACKGROUND

Indoxacarb is a new insecticide under review for inclusion in Annex I of Council Directive 91/414/EEC\(^4\). On the basis of the evaluation report established by The Netherlands as Rapporteur Member State (RMS), the substance was discussed in the working group “Plant Protection Products - Evaluation” of the Standing Committee on Plant Health.

The substance causes damage to haemoglobin, resulting in an increased turnover of red blood cells. At low exposure levels, some fluctuations in isolated red blood cell parameters were observed, which are of unclear biological significance and may be incidental. Based on a weight of evidence consideration of all available short and long term studies an overall NOEL of 10 ppm or 0.6 mg/kg was proposed for the effects on blood parameters in rats. The U.S. EPA has recently reached a similar conclusion and selected 40 ppm as a NOEL.

Source documents made available to the Committee:

1. Indoxacarb: EPA fact sheet, conditional registration, 30 October 2000

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I. Question 1

Can the Committee comment on the NOEL for effects on red blood cells in rats?

Changes in some RBC parameters (RBC count, hematocrit, haemoglobin level) after repeated indoxacarb treatment were generally slight (<10% and within the reference values, although sometimes significantly lower than control values) and not accompanied by a significant reticulocytosis. The observed haemosiderin deposits in spleen and liver, and spleen and bone marrow hyperplastic response should be considered secondary physiological response to the increased RBC turnover. The overall picture is that of a mild haemolytic effect, possibly due to oxidative stress on RBCs, with a very shallow dose response curve. While the Committee concluded that a clear NOEL could not be established, the absence of reticulocytosis and the slight changes (<10%) in treated animals with values mostly within the range of normal values lead the Committee to consider that these effects are non adverse up to 40 ppm.
Scientific background on which the opinion is based:

I.1. Haemopoiesis and anemia

Haematopoiesis is the process by which the formed elements of the blood are produced. The process is regulated through a series of steps beginning with the pluripotent haematopoietic stem cell. This occurs under the regulatory influence of growth factors and hormones, such as erythropoietin (EPO) for red cell production. The fundamental stimulus for EPO production, mainly by peritubular capillary lining cells within the kidney, is the availability of O\textsubscript{2} for tissue metabolic needs. Impaired O\textsubscript{2} delivery to the kidney can result from a decreased red cell mass (anaemia), impaired O\textsubscript{2} loading of the haemoglobin molecule (hypoxemia), or, rarely, impaired blood flow to the kidney (renal artery stenosis). EPO governs the day-to-day production of red cells. When the haemoglobin concentration falls below 100 to 120 g/L (10 to 12 g/dL), plasma EPO levels increase logarithmically in inverse proportion to the severity of the anaemia. Generally, anaemia is recognised in the laboratory when a patient's haemoglobin level or hematocrit is reduced below an expected value (the normal range). The haemoglobin concentration in adults has a Gaussian distribution. The mean hematocrit value for adult males is 47% (± SD\textsuperscript{5} 7) and that for adult females is 42% (± SD 5). Any individual hematocrit or haemoglobin value carries with it a likelihood of associated anaemia. Suspected low haemoglobin or hematocrit values are more easily interpreted if there are historic values for the same patient for comparison.

Because of the intrinsic compensatory mechanisms that govern the O\textsubscript{2}-haemoglobin dissociation curve, the gradual onset of anaemia, particularly in young patients, may not be associated with signs or symptoms until the anaemia is severe [haemoglobin <70 to 80 g/L (7 to 8 g/dL)]. With chronic anaemia, intracellular levels of 2,3-bisphosphoglycerate (BPG) rise, shifting the dissociation curve to the right and facilitating O\textsubscript{2} unloading. This compensatory mechanism can maintain normal tissue O\textsubscript{2} delivery in the face of a 20 to 30 g/L (2 to 3 g/dL) (i.e.: 15-20% decrease) deficit in haemoglobin concentration.

A routine complete blood count includes the haemoglobin, hematocrit, and red cell indices: the mean cell volume (MCV), mean cell haemoglobin (MCH), and mean concentration of haemoglobin per volume of red cells (MCHC). Other important information is provided by the reticulocyte count and measurements of iron supply including the serum iron, the total iron-binding capacity (an indirect measure of the transferrin level), and serum ferritin. Reticulocytes are red cells that have been recently released from the bone marrow. Normally, the reticulocyte count ranges from 1 to 2% and reflects the daily replacement of 0.8 to 1.0% of the circulating red cell population. A correctly interpreted reticulocyte count provides a reliable measure of red cell production.

In general, if the EPO and erythroid marrow responses to moderate anaemia [haemoglobin <100 g/L] are intact, the red cell production rate increases to two to three times above normal within 10 days following the onset of anaemia.

The three major classes of anaemia are: (1) marrow production defects (hypoproliferation), (2) red cell maturation defects (ineffective erythropoiesis), and (3) decreased red cell survival (blood loss/hemolysis). A hypoproliferative anaemia is typically seen with a low reticulocyte production index together with little or no change in red cell morphology (a normocytic, normochromic anaemia). Maturation disorders typically have a slight to moderately elevated reticulocyte production index that is

\textsuperscript{5} Standard Deviation
accompanied by either macrocytic or microcytic red cell indices.

The loss of red cells either through hemorrhage or, less commonly, through premature destruction of the red cells (haemolysis) may cause anaemia. Haemolysis or blood loss leads to an increase in red cell production, which is clinically manifested by an increase in reticulocytes.

An elevated reticulocyte count in the patient with anaemia is the most useful indicator of hemolysis, reflecting erythroid hyperplasia of the bone marrow; biopsy of the bone marrow is often unnecessary.

RBC may be prematurely removed from the circulation by macrophages, particularly those of the spleen and liver (extravascular lysis), or, less commonly, by disruption of their membranes during their circulation (intravascular haemolysis). Both mechanisms result in increased heme catabolism and enhanced formation of unconjugated bilirubin, which is normally conjugated by the liver and excreted. Elevated bilirubin levels, mainly unconjugated, are a clinically useful laboratory parameter of increased RBC destruction.

Haptoglobin is a globulin that is present in high concentration (~1.0 g/L) in the plasma. It binds specifically and tightly to the globin in haemoglobin. The haemoglobin-haptoglobin complex is cleared within minutes by the mononuclear phagocyte system.

Intravascular haemolysis results in the release of haemoglobin into the plasma. If the haptoglobin-binding capacity of the plasma is exceeded, free haemoglobin passes through renal glomeruli. This filtered haemoglobin is reabsorbed by the proximal tubule, where it is catabolised in situ, and the haeme iron is incorporated into storage proteins (ferritin and hemosiderin). The presence of haemosiderin in the urine, detected by staining the sediment with Prussian blue, indicates that a significant amount of circulating free haemoglobin has been filtered by the kidneys (Harrison’s, 2001).

I.2. Experimental studies with indoxacarb

Haematological effects have been observed in rats and dogs, but not in mice. Four 90-day and one 2-year rat, and one 90-day and one 1-year dog studies were available. Short-term and long-term studies indicate that the targets of indoxacarb toxicity in rats and dogs are the red blood cells (RBC). This results in a mild haemolytic anaemia which appears as early as 3 weeks after the beginning of treatment with little evidence of progression at later time-points. Indeed, in the two-year rat study haematological data in treated groups did not differ from those of the control group at 18 and 24 months. In a 90-day dietary study followed by a 21 day recovery period the haematological effects fully recovered. The dose-response curves were always very shallow and a clear NOEL could not be identified. Decrease higher than 10% below controls of more than one RBC mass parameters (RBC count, hematocrit, haemoglobin level) were observed at 40-50 ppm and above in rats, and at 40-80 ppm and above in dogs. In most cases, however, these values were within the reference range. Increased haemosiderin deposits were observed at 20 ppm and above in rats, and at 400 ppm and above in dogs. In general, deposits in the spleen occurred at lower doses than in liver. Spleen and bone marrow hyperplasia occurred at 50 ppm and above in the 90-day rat studies, at 125 ppm in the interim sacrifice (1 year) but not at termination of the 2-year rat study. In dogs, this was observed at 80 ppm and above. Slight increase in serum bilirubin was observed only in certain occasions at 200/250 ppm in rats and at 160 ppm in dogs. Increased reticulocyte counts were observed in certain studies at 125 ppm in rats and at 640 ppm in dogs. The
reductions of hematocrit, RBC count and haemoglobin content were generally less than 20% in rats at the maximum doses of 250 ppm (16 mg/kg bw/day) and 125 ppm (9.5 mg/kg bw/day) in males and females, respectively. In dogs, reductions were somewhat higher (up to 28%) at 640-1280 ppm (17 and 34 mg/kg bw/day, respectively) in both males and females. In some studies methaemoglobinemia was also measured and found to be slightly increased. This, and the presence of Heinz Bodies, are consistent with the hypothesis of an oxidative stress applied to the RBCs by administration of indoxacarb. This effect is likely due to the metabolite trifluoromethoxyaniline (IN-P0036), after N-hydroxylation (see opinion on question 2).

In addition, experimental data in vitro suggest that haemoglobin in the rat and dog is generally somewhat more sensitive than human haemoglobin to oxidative stress (Harvey and Kaneko, 1976). For instance, human RBCs are 3-4 times more resistant than rat RBCs to the haemolysis caused by dapsone hydroxylamine (likely via oxidative stress) (McMillan et al., 1995). However, this is not necessarily true for all compounds (Harvey and Kaneko, 1976).

I.3 Conclusions

The Committee is of the opinion that a number of points should be considered when assessing the relevance of haematological findings in animals treated with indoxacarb. It is clear that in almost all studies in rats and dogs a decrease in one or more RBC parameters was sometimes observed at low doses and available evidence (the presence of an aniline metabolite, the haemosiderin deposits in spleen and liver, spleen and bone marrow hyperplastic response, increased serum bilirubin levels) indicates that this is due to an accelerated RBC turn over possibly because of oxidative stress. Changes in some RBC parameters (RBC count, hematocrit, haemoglobin level) were generally slight (<10% and within the reference values, although sometimes significantly lower than control values) and not accompanied by a significant reticulocytosis. In fact, this was only observed (in some studies) at the highest doses indicating that tissue oxygenation was not affected at lower doses. The haemosiderin deposits in spleen and liver, and spleen and bone marrow hyperplastic response should be considered to be secondary physiological responses to the increased RBC turn over. The very shallow dose-response curve also indicates that the compensatory mechanism of the haemopoietic system was not overcome (except at high doses in the dog) and the effect of indoxacarb in rats and dogs can be described as a compensated haemolytic effect. In conclusion, the overall picture is that of a mild haemolytic effect with a very shallow dose response curve. While the Committee concluded that a clear NOEL could not be established, the absence of reticulocytosis, the slight changes (<10%) in treated animals with values mostly within the range of normal values may lead to the view that these effects are non adverse up to 40 ppm. Note should also be taken of the lack of effects in mice and of the apparent lower sensitivity of human RBCs to oxidative stress.
II. Question 2

The Committee is requested to comment on the adequate basis for the derivation of an Acute Reference Dose (ARfD) for indoxacarb.

The Committee is of the opinion that the general and non-specific signs of toxicity observed in the acute neurotoxicity study in rats (NOAEL 12.5 mg/kg) can be used as a basis for the derivation of an ARfD.

Scientific background on which the opinion is based:

Indoxacarb is slowly absorbed since peak plasma concentration was reached 6.8-8 hours after dosing, and slowly eliminated with plasma half-lives of the indanone moiety of 35-45 hours in males and 52-59 hours in females, depending on the dose, whereas those of the trifluoromethoxyphenyl moiety were 92-96 and 114-188 hours, respectively. In red blood cells half-lives were somewhat longer. At high dose saturation occurred, since the plasma AUC\(^6\) of the indanone moiety after 5 mg/kg bw was 80(males)-135 (females) μg/g x h and after 150 mg/kg bw it was only 536-785 μg/g x h. In red blood cells AUC were not significantly different. On the other hand, red blood cell AUC of the trifluoromethoxyphenyl moiety was much higher after both the low dose (1519-2627 μg/g x h, males and females respectively) and the high dose (7656-16648 μg/g x h). The trifluoromethoxyphenyl moiety gives the metabolite trifluoromethoxyaniline (IN-P0036) which, after N-hydroxylation, is the likely metabolite causing damage to red blood cells. A notable sex difference is also reflected in the higher sensitivity of female rats to the toxic effects of indoxacarb. When rats were treated with multiple doses of \(^{14}\)C-indoxacarb (5 mg/kg bw per day), the radiolabel reached a steady-state concentration in red blood cells after about two weeks. The concentration was about 8 fold higher than that reached after a single dose. The radiolabel is most probably mainly associated with trifluoromethoxyaniline (IN-P0036), which causes oxidative damage to red blood cells.

Toxicity by indoxacarb in rats and dogs is characterised by haematological effects after repeated dosing and general non-specific signs of toxicity (reduced body weight and body weight gain, decreased food intake, alopecia etc). These general signs were observed also in dams in rat teratogenicity studies with an overall NOAEL of 2 mg/kg bw. As such these are not adequate for the derivation of an ARfD. Indoxacarb has an oral LD\(^7\)\(_{50}\) in rats of about 268 mg/kg bw. Indoxacarb did not cause increased incidence of tumours, was not genotoxic, not toxic to reproduction, not teratogenic and was not an irritant. Indoxacarb was found to be a skin sensitizer.

Limited data in dogs indicate that the haematological effects did not occur after one week of daily treatments with 80 ppm indoxacarb in the diet (equal to 3 or 2 mg/kg bw in males and females respectively).

In an acute neurotoxicity study in rats, alteration of some FOB (functional observational battery) parameters (males) and in motor activity (females) were observed at the highest dose associated with, and possibly due to, general toxicity (reduced body weight and body weight gain, decreased food intake, alopecia etc). Reduced body weight gain in males, and reduced food consumption and alopecia in females were also observed in the

\(^6\) Area Under the Curve
\(^7\) Lethal Dose, Median
mid dose. The NOAEL in this study was 25 mg/kg bw in males and 12.5 mg/kg bw in females.

II.1. Conclusions

Based on toxicokinetic and toxicological data it is unlikely that haematological effects occur after a single dose of indoxacarb. The Committee is of the opinion that the general and non-specific signs of toxicity observed in the acute neurotoxicity study in rats (NOAEL 12.5 mg/kg) can be used as a basis for the derivation of an ARfD.

F. REFERENCES


Acknowledgements

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