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Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective

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Abstract

Haemolytic anaemia is often induced following prolonged exposure to chemical substances. Currently, under EU Council Directive 67/548/EEC, substances which induce such effects are classified as dangerous and assigned the risk phrase R48 'Danger of serious damage to health by prolonged exposure.' Whilst the general classification criteria for this endpoint are outlined in Annex VI of this Directive, they do not provide specific information to assess haemolytic anaemia. This review produced by the EU Working Group on Haemolytic Anaemia provides a toxicological assessment of haemolytic anaemia and proposes criteria that can be used in the assessment for classification of substances which induce such effects. An overview of the primary and secondary effects of haemolytic anaemia which can occur in rodent repeated dose toxicity studies is given. A detailed analysis of the toxicological significance of such effects is then performed and correlated with the general classification criteria used for this endpoint. This review intends to give guidance when carrying out an assessment for classification for this endpoint and to allow for better transparency in the decision-making process on when to classify based on the presence of haemolytic anaemia in repeated dose toxicity studies. The extended classification criteria for haemolytic anaemia outlined in this review were accepted by the EU Commission Working Group on the Classification and Labelling of Dangerous Substances in September 2004.

Keywords: Haemolytic anaemia; Repeated dose; Classification; Erythrocytes; Haematopoietic system; Regulatory toxicology; Haemosiderin; Methaemoglobin; Council Directive 67/548/EEC

1. Introduction

Within the European Union, protection of human health and the environment from the dangerous effects of hazardous chemicals is achieved in part by classification and labelling of such chemicals. The classification has a large number of downstream consequences within EU legislation. The requirements for the classification and labelling of substances are described in Annex VI of Council Directive 67/548/EEC and its amendments (EU, 1967). For each endpoint such as carcinogenicity, irritation or acute toxicity classification criteria are provided which include dose limits and/or effect limits. Substances which induce adverse effects following prolonged exposure such as that observed in repeated dose toxicity studies at dose levels below specified limits are classified and labelled as 'Danger of serious damage to health by prolonged exposure' and assigned the risk

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phrase R48. The decision to classify for this endpoint depends on several factors including the severity of the effect, the reversibility of the effect, magnitude of the effect and the possibility of a serious impact on health after prolonged exposure. Further information on classification and labelling in the EU can be found at: http://ecb.jrc.it/classification-labelling/.

Haemolytic anaemia is one effect often found in repeated-dose toxicity studies. However, it is often unclear when the primary and secondary effects of haemolytic anaemia warrant classification of a substance with R48, which, in part, is due to the non-specific nature of the criteria outlined in the aforementioned Directive. This has resulted in many discussions between the member states and industry in EU Commission Working Groups on the Classification and Labelling of Dangerous Substances meetings. To resolve this problem, a working group was formed to propose specific criteria for the classification of substances inducing haemolytic anaemia with R48.

To facilitate consistent classification of substances causing haemolytic anaemia, a description of the primary and secondary effects of haemolytic anaemia is provided including a non-exhaustive review of the possible toxicological indicators of haemolytic anaemia. Classification criteria that can be used to aid in the classification of a substance for this endpoint under the EU system are proposed in Appendix A.

The proposed criteria were accepted by the EU Commission Working Group on the Classification and Labelling of Dangerous Substances in the meeting of September 2004, JRC, Ispra, Italy.

2. Overview of anaemia

2.1. Anaemia

Anaemia is usually defined as a reduction of the haemoglobin concentration, red blood cell count or packed cell volume to below normal levels. As a result, the oxygen carrying ability of the blood is reduced. The causes of anaemia are divided into failure of red cell proliferation, defective maturation of red blood cells, haemolysis, and blood loss. Exogenous agents can induce anaemia by some of these different mechanisms. Classification of chemicals that cause anemia by other mechanisms, e.g. blood loss or direct toxicity to the bone marrow resulting in decreased bone marrow production of red blood cells, are not considered in this review.

Symptoms of anaemia, depend on three factors: the causative disorder, the degree of erythrocyte destruction and the reduction of oxygen-carrying capacity. The symptoms can vary with the severity of anaemia from decreased physical activity, fatigue, headache, faintness, increased sensitivity to cold, tinnitus, black spots before the eyes, irritability, lack of power of concentration, fever, pallor, dyspnoea, tachycardia, systolic murmurs, cyanosis, nausea, vomiting and abdominal pain to death.

Anaemia can cause serious damage to health. For example anaemic older people without clinical disease have an increased risk of mortality (Izaks et al., 1999) and there is an association between anaemia and greater physical decline (Penninx et al., 2003). There is increasing evidence that anaemia adversely affects the health and life quality of individuals (Baker and DeMaeyer, 1979). It should be kept in mind that weakness, fatigue or lassitude may accompany slowly developing chronic anaemia, in the absence of other symptoms and reduced exercise tolerance in patients.

2.2. Haemolytic anaemia

Haemolytic anaemia is an anaemia caused by accelerated destruction of mature red cells outside the bone marrow or a consequence of the destruction of imperfectly formed red cells. Haemolytic disorders can be classified as intracorpuscular defects, which are mainly hereditable disorders (e.g. hereditary spherocytosis, G6PD-deficiencies, thalassemia, sickle cell disease, etc.) and extracorpuscular defects (caused by e.g. infectious, chemical and physical agents and auto-antibodies). Haemolysis may be intravascular and extravascular or a combination of both.

Classical signs of haemolytic anaemia are hyperbilirubinemia often accompanied by jaundice, increased excretion of haemoglobin breakdown products (haemoglobin or haemosiderin) in the urine and stools and evidence of appropriate bone marrow erythropoietic response (reticulocytosis).

Haemolytic anaemia induced by chemical agents may be more detrimental for people with hereditable haemolytic disorders and for people with pre-existing anaemia. Vulnerable subgroups for haemolytic anaemia are individuals already low in haemoglobin status including women of menstruating age (Barr et al., 1998), children (Tapiero et al., 2001) and the elderly (Beutler, 1995).

2.2.1. Intravascular haemolysis

During intravascular haemolysis, the red blood cell is lysed within the general circulation so that haemoglobinemia is pronounced and hyperbilirubinemia may occur. Free haemoglobin in the circulation is complexed by haptoglobin and metabolised in the reticuloendothelial system. If the binding capacity of haptoglobin is exceeded, haemoglobin appears in the plasma where it is degraded, and the haem, which is liberated, binds to haemopexin. When the binding capacities of haptoglobin and haemopexin are saturated, free haemoglobin appears in the plasma where it is rapidly oxidized to methaemoglobin (MetHb), dissociated, and the haem binds to albumin to form methaemalbumin (Erslev and Beutler, 1995). Remaining unbound (met)haemoglobin is filtered into the primary urine and reabsorbed in the renal proximal tubules, where the iron is extracted and incorporated into haemosiderin. Haemoglobinuria occurs when the haemoglobin concentration exceeds the resorption capacity of the proximal renal tubules and is indicative of extensive intravascular haemolysis. Haemosiderin is excreted in urine but may not be detectable in the urine for several days after