Regulatory Toxicology and Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)acute toxicity profile in a high quality dataset

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ARTICLE INFO

Article history:
Received 3 January 2014
Available online xxxx

Keywords:
3Rs
Repeated dose
Sub-chronic
REACH
Chemicals legislation
Alternatives
Sub-acute

ABSTRACT

A survey conducted on the EU Notification of New Substances (NONS) database suggested that for industrial chemicals with a profile of low toxicity in (sub)acute toxicity tests there is little added value to the conduct of the 90-day repeated dose study. Avoiding unnecessary animal testing is a central aim of the EU REACH chemicals legislation; therefore we sought to verify the profile using additional data. The OECD's eChemPortal was searched for substances that had both a 28-day and a 90-day study and their robust study summaries were then examined from the ECHA CHEM database. Out of 182 substances with high quality 28-day and 90-day study results, only 18 reported no toxicity of any kind in the (sub)acute tests. However, for 16 of these there were also no reported signs of toxicity at or close to the limit dose (1000 mg/kg bw/d) in the 90-day study. Restricting the 'low (sub)acute toxicity in a high quality dataset' profile to general industrial chemicals of no known biological activity, whilst allowing irritant substances, increases the data set and improves the prediction to 95% (20 substances out of 21 substances). The low toxicity profile appears to be of low prevalence within industrial chemicals (10–15%), nevertheless, avoidance of the conduct of a redundant 90-day study for this proportion of the remaining REACH phase-in substances would avoid the use of nearly 50,000 animals and save industry 50 million Euros, with no impact on the assessment of human health.

1. Introduction

All new and existing chemical substances that are manufactured or imported in the European Union must now be registered under EU chemicals legislation REACH (Regulation (EC) No. 1907/2006). The difference between REACH and previous EU chemicals legislation is that the information requirements for existing chemicals (so called phase-in substances) are the same as for new (non-phase-in) substances. Companies had to register all the substances they manufacture or import in quantities above 1000 tonnes per year by 1 December 2010. All the substances they manufacture or import in quantities above 100 tonnes per year were also registered by 1 June 2013. A complete data package for a REACH chemical registration at these tonnages can involve the conduct of at least 10 different animal studies and can cost between 800 and 1600 k Euros (for Annex IX and X, respectively) (based on the most recent figures available; Fleischer, 2007).

An example of the heavy burden of safety information required under REACH is that for substances manufactured or imported at levels of 100 tonnes per year or above (Annex IX requirements), the results of both a 28-day and a 90-day repeated dose toxicity study in rodents is required. This requirement was based on the general assumption that the No Observed (Adverse) Effect Level (NO(A)EL) of a substance decreases as the length of the study increases. For substances that are produced in high quantities it was therefore decided that a 90-day study should be required in addition to the 28-day study. However, in order to reduce unnecessary animal testing, for those substances that have neither a 28-day nor a 90-day study, it is permitted to provide the results of the 90-day study only (REACH Annex IX 8.6.2, column 1). Table 1 outlines the repeated dose and reproductive toxicity requirements of substances being registered under REACH.

Not all existing substances will have the necessary information requirements and it is quite possible that many substances will actually have the results of the 28-day but not the 90-day study. In fact, a review by the European Commission estimated that 93% of relevant substances would not have the 90-day test prior to

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http://dx.doi.org/10.1016/j.yrtph.2014.04.008
0273-2300/

Please cite this article in press as: Taylor, K., et al. The added value of the 90-day repeated dose oral toxicity test for industrial chemicals with a low (sub)-acute toxicity profile in a high quality dataset. Regul. Toxicol. Pharmacol. (2014), http://dx.doi.org/10.1016/j.yrtph.2014.04.008

Table 1
REACH requirements for repeated dose and reproductive toxicity studies.

Annex	VII	VIII	IX	X
Tonnage	1 tonne or greater	10 tonnes or greater	100 tonnes or greater	1000 tonnes or greater
Repeated dose toxicity	None	28 day (most appropriate route)	28 day (unless already conducted or the 90 day is proposed) 90-day study should be proposed (most appropriate route)	28 day (unless already conducted or the 90 day is proposed) 90-day study should be proposed (most appropriate route) Longer term studies may be proposed if serious or severe toxicity effects of particular concern were observed in the 28-day or 90-day study for which the available evidence is inadequate for toxicological evaluation or risk characterisation A carcinogenicity study may be proposed if there is evidence from the repeated dose study(s) that the substance is able to induce hyperplasia and/or preneoplastic lesions (and there is wide dispersive use of the substance or frequent human exposure)
Reproductive toxicity	None	Screening study (OECD TG 421)	Prenatal developmental toxicity should be proposed (most appropriate route) Two generation reproductive toxicity study should be proposed, if the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues	Prenatal developmental toxicity should be proposed (most appropriate route) A second species prenatal developmental toxicity should be considered and proposed (most appropriate route) Two generation reproductive toxicity study, unless already provided or proposed

REACH (Pedersen et al., 2003). This is because previous regulatory regimes such as the predecessor to REACH, the Dangerous Substances Directive (Directive 67/548/EEC Annex VIIA) and the voluntary US High Production Volume Challenge Programme only required the 28-day study within the basic data package. Many REACH registrants are therefore now in the position of having to submit 'proposals' to conduct 90-day studies on their substance if the test cannot be waived for other reasons (read across, exposure based arguments, etc.; see Annex XI of REACH for adaptations to the standard testing regime).

Testing proposals must be submitted, not the test result, because REACH says that a proposal for tests required under Annex IX or X, such as the 90-day study, must be first evaluated by the agency responsible – the European Chemicals Agency (ECHA) – before it is conducted. This is a test reduction measure put in place to allow third parties to notify ECHA that they have existing information on the substance, thereby avoiding duplicate animal studies. Testing proposals are published on the ECHA website for a 45 day comment period and then a decision is made via a bureaucratic process that can take at least a year.

In this period of intense data collection to satisfy REACH requirements it is crucial to seek to maximise efficiency, not only of animals but of cost to the industry at large. Despite the animal reduction measures written into the legislation, within a complete REACH submission for which the results of several toxicological studies of different durations and covering a range of endpoints are required, it is entirely possible that there will be tests that are less useful than others. Due to the similar nature of the repeated dose tests – the only difference being duration of the dosing – it is feasible that there may be some element of duplication to the conduct of both the 28-day and the 90-day study for example.

Identifying studies that add little in terms of information on the hazardous properties of the substance, and are in effect 'redundant', is a relatively easy way to reduce animal testing and costs to industry, without adversely impacting on the level of protection of human or environmental health. Examples of where this approach has proved fruitful include a review of the need for dermal acute toxicity studies for industrial chemicals and pesticides (Creton et al., 2010) and a review of the need for single dose acute toxicity studies for medicinal compounds (Robinson et al., 2008) and for industrial chemicals (Chapman et al., 2010) all by the UK National Centre for the 3Rs, a review of the need for the second generation in reproductive toxicity studies by the Dutch National Institute for Public Health and the Environment (Janer et al., 2007), and a review of the need for carcinogenicity studies for medicinal products by the German Federal Institute for Drugs and Medical Devices (Friedrich and Olejniczak, 2011) and also by the US drug industry (Sistare et al., 2011).

Based on their experience with the Notification of New Substances (NONS) system – the chemical registration system that predated REACH – some members of the UK Competent Authority for REACH [the Health and Safety Executive (HSE)] felt that there may be redundancy in the 90-day repeated dose toxicity study. This is because when they reviewed NONS dossiers with low toxicity in the 28-day study they consistently found that the 90-day study also demonstrated low toxicity. To demonstrate their hypothesis, the HSE performed a systematic analysis of the NONS database, which was not publicly available at the time. They found that out of 110 substances with results for both 28-day and 90-day studies via the same exposure route, 17 substances (15%) were identified that had a NO(A)EL close to or greater than 1000 mg/kg bw/d in the 28-day study. In all these substances the 90-day NO(A)EL was also equivalent to or higher than the limit dose of 1000 mg/kg bw/d. The limit dose of 1000 mg/kg bw/d is a pragmatic value introduced to prevent the use of excessive dose levels in toxicity studies, which would be likely to result in effects of no relevance to the human risk assessment. The results of their analysis therefore strongly suggested that there was no added value to the conduct to the 90-day study in these situations, with no margin of error. As a precautionary approach they recommended that all acute endpoints including acute toxicity (by any route), mutagenicity, skin sensitisation and skin and eye irritation should also be negative for the substance to satisfy the 'low toxicity profile'. However, this would have reduced their dataset to 14 substances with obviously no improvement to be gained in predictivity.

The HSE presented their results to the Member State Committee (MSC) at the ECHA in January 2011 (HSE, 2011). The MSC is ECHA's committee that agrees on the need for new toxicity tests when conducting compliance checks of registration dossiers and testing proposals made by registrants who have already identified that they have a data gap. The MSC members expressed interest in

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