Regulatory Toxicology and Pharmacology 90 (2017) 258–261

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: An update

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ARTICLE INFO

Article history:
Received 19 June 2017
Received in revised form
8 September 2017
Accepted 13 September 2017
Available online 15 September 2017

Keywords:
Repeated dose
Low toxicity
REACH
Sub-acute
Oral
European Chemicals Agency

ABSTRACT

A previous retrospective analysis of substances in the ECHA CHEM database concluded that, for industrial chemicals with a 'low (sub)acute toxicity profile', further testing in the 90-day study is unlikely to change this profile (Taylor et al., 2014). We have further tested this hypothesis by assessing the outcome of substances with testing proposals for which a prediction was made in that paper that the NOAEL based on the 90-day study would be 1000 mg/kg bw/d. Indeed, for seven out of ten substances for which data was available, the profile was shown to be held. For three substances, the reduced NOAEL was explained by renal effects in the rats, two of which had been seen in the 28-day study but had been dismissed by the study submitter. We conclude that the low toxicity profile will be even more protective if the NOEL is used from the 28-day study and an independent expert view is taken of the human relevance of any effects reported in the 28-day study.

1. Introduction

A previously published retrospective analysis of substances in the ECHA CHEM database (Taylor et al., 2014) concluded that, for industrial chemicals with a 'low (sub)acute toxicity profile', further testing in the 90-day study is unlikely to change this profile. We defined substances with a low (sub)acute toxicity profile as those having:

- Experimental data equivalent to OECD 407 (28-day repeated dose oral study) with a reported NOAEL of 1000 mg/kg bw/d or higher
- Experimental data that do not meet the criteria for classification for mutagenicity, skin sensitisation or acute toxicity by any route
- No additional evidence based on physical chemical properties, structure or use to suggest that the substance could be biologically active

For the original analysis, we required that the absence of toxicity for these endpoints needed to be supported by high quality experimental data on the substance itself, up to the limit dose, where relevant.

Our original analysis identified 21 substances that met this definition out of only 182 substances with data from both 28-day and 90-day studies in the European Chemicals Agency (ECHA) ECHACHEM database (see www.echa.europa.eu). Of these, 20 (i.e. 95%) had a 90-day study that also reported a NOAEL of 1000 mg/kg bw/d or higher. It was concluded therefore, that performing a 90-day study for low (sub)acute toxicity substances did not add any useful information to the overall toxicological dataset.

This analysis was in effect a retrospective validation of the 'low (sub)acute toxicity profile' hypothesis that had been originally proposed by the UK Health and Safety Executive (who had initially found the profile fitted 16 out of 18 substances registered under the Notification of New Substances (NONS) system) (see Taylor et al., 2014).

However, due to the low number of substances available upon which to test the hypothesis we felt that more validation would be helpful. To this end, in the same paper (*4.10. Prediction of results from substances with 90-day studies proposed*) we identified 14 substances which we considered may meet the low toxicity criteria out of 114 substances for which testing proposals for a 90-day study had been submitted to ECHA by the registrants. This would, in

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https://doi.org/10.1016/j.yrtph.2017.09.018

effect, be a prospective validation of the hypothesis since the outcome was not yet known. Naturally some time has had to pass to ensure that these substances now have the 90-day robust study summaries disseminated in the ECHACHEM database. In this communication, we are now, however, pleased to be able to present the results of the outcome of the proposed testing and a review of the robustness of the 'low (sub)acute toxicity profile' hypothesis.

2. Results

Of the 14 substances, 90-day studies were available for 12, two of which were performed using read-across substances. For the remaining two substances, the tonnage band at which the substance was registered had changed, meaning that a 90-day study was no longer required. Of the ten 90-day studies performed using the registered substances, all were performed at dose levels up to the limit dose of 1000 mg/kg bw/d. Details of the fourteen 'low (sub)acute toxicity profile' substances identified in the previous paper with testing proposals for a 90-day study are presented in Table 1.

We correctly predicted that five of ten substances with actual 90-day test data would not have a NOAEL less than 1000 mg/kg bw/d in the longer-term study (EC 641-136-6; EC 426-040-2; EC 203-838-7; EC 211-074-0; EC 203-326-3). This extends to seven out of 12 if the two substances with read across data are included (EC 402-140-1; EC 404-370-8). For one substance, we were over cautious and did not predict a NOAEL less than 1000 mg/kg bw/d, however one was reported (**EC 271-237-7**). We had considered that the marginal effect on red blood cell parameters noted at 300 mg/kg bw/d for males in the 28-day study could have resulted in similar or more severe effects in the 90-day, however no such effects were reported. For one substance (**EC 204-111-7**), we were wise to exercise caution in ascribing it to the low (sub)acute toxicity profile. A NOAEL of 15 mg/kg bw/d was reported for the 90-day study for males on the basis of renal effects. Although renal effects were also reported in the 28-day study in males at dose levels of 150 and 1000 mg/kg bw/d, these findings were considered not to be of relevance to the human risk assessment by the test submitter when deriving the NOAEL.

For three substances, effects were seen in the 90-day study that resulted in the data submitter suggesting a lower NOAEL than we had predicted (EC 230-991-7; EC 432-070-7; EC 480-370-1). For two of these, effects had been seen in the 28-day study that were disregarded by that study submitter when setting the NOAEL:

For **EC 432-070-7**, a number of deaths were reported in the 90-day study with this substance, the majority of which were attributed to dosing error. Transient signs of toxicity (hypoactivity, piloerection) were reported in females dosed at 1000 mg/kg bw/d. A NOAEL of 250 mg/kg bw/d was determined for this study based on changes in clinical chemistry and haematological parameters, pathology of the kidneys, bladder and stomach at 1000 mg/kg bw/d. The 28-day study for this substance reported signs of toxicity and some statistically significant changes in clinical chemistry and haematological parameters in rats administered 1000 mg/kg bw/d; findings were discounted in derivation of the NOAEL.

In the 90-day study of **EC 230-991-7**, one male administered the highest dose level of 1000 mg/kg bw/d was sacrificed *in extremis* on Day 33; the death is attributed to treatment but this pattern of mortality is not indicative of an effect of treatment. A number of effects at 300 and 1000 mg/kg bw/d were attributed to urolithiasis and obstructive nephropathy and a NOAEL of 100 mg/kg bw/d was therefore reported. In the 28-day study a NOEL of 200 mg/kg bw/d was based on isolated minor and reversible changes in clinical chemistry and urinalysis at 1000 mg/kg bw/d; findings are consistent with those seen in the 90-day study.

For **EC 480-370-1**, however, no effects on the kidneys (or other organ) were noted in the 28-day study, yet the 90-day study threw up some renal effects, resulting in a NOAEL of 100 mg/kg bw/d being reported for this substance.

3. Conclusion

We reviewed the 90-day study results of 14 substances potentially meeting the low toxicity profile predicted in Taylor et al. (2014). Of the 14 substances identified, ten now have 90-day studies available. Of these studies, six reported a clear NOAEL of 1000 mg/kg bw/d, thereby fitting the original hypothesis. (A further two reported read across data that also supports the original hypothesis). The four remaining substances have 90-day NOAELs reported to be < 1000 mg/kg bw/d; for three substances, the effects driving the 90-day NOAEL had also been reported at the same or similar dose levels in the 28-day study but had been discounted as not relevant for derivation of the NOAEL. For one of these (**EC 204-111-7**), which showed the most notable effects in the 90-day study out of these substances, we had been correct to register hesitation in predicting that this substance fitted the low toxicity profile. For the single remaining substance (**EC 480-370-1**), the renal effects in male rats driving the 90-day NOAEL were not apparent in the 28-day study.

Those substances for which the prediction was not strictly met were all characterised by renal effects seen in the rats, particularly males. Male rat-specific kidney effects are relatively common and are often dismissed as not being of relevance to humans. This perhaps explains why there was some inconsistency in the test submitters as to the consideration of the NOAEL. As explained in Taylor et al. (2014), there was limited analysis on our part of the significance of the biological results and we did not adjust the test submitter's decision on the NO(A)EL based on the 28-day study result.

In the interests of keeping the prediction model simple it is therefore perhaps advisable that any findings in 28-day studies (whether considered adverse or not) should be taken into account and the profile is considered met if there is a NOEL of 1000 mg/kg bw/d or greater in the 28-day study (rather than a NOAEL).

Had we applied this more cautious approach we would not have ascribed the low-toxicity profile to a further two substances out of the 14 substances. This would have reduced the dataset further and been slightly too sensitive (one of the four with some effects in the 28-day study was subsequently ascribed a NOAEL of 1000 mg/kg bw/d from the 90-day study). However, it would have made the prediction correct in five out of six cases (83%) or seven out of eight (88%) if the read across results are accepted.

A recent additional analysis of the ECHCHEM database by others has also provided support to the profile (Luechtefeld et al., 2016). Just using a data mining tool, they found that out of 121 substances fitting the low (sub) acute toxicity profile we suggested in Taylor et al. (2014), 70.2% also had NOAEL's greater than 1000 mg/kg bw/d based on the 90-day study. They did not do any expert screening of the data however and used the NOAEL in the 28-day rather than the NOEL (both now recommended following our follow up analysis). Nonetheless it is clear that their analysis supports our hypothesis. Furthermore, they found that if the assessment factor of 3 is applied to the 28-day study to derive a 90-day DNEL (Derived No Effect Level) (ECETOC, 2010) then only 8.3% of 122 substances had an actual 90-day based NOAEL below this.

We believe these further validations of the 'low (sub)acute toxicity profile' hypothesis should encourage chemical regulators to investigate the option to waive the 90-day study in certain circumstances. We propose that consideration is given to amending REACH, and similar legislation, such that, where both a 28-day and

Table 1
The results of substances with 90-day testing proposals predicted in Taylor et al. (2014).

EC No.	28-day NOAEL (rat, oral) result	Comments on the 'low toxicity profile'	Prediction made in Taylor et al. (2014)	90-day NOAEL (rat, oral) result	Comments on the 90-day study result
480-370-1	1000 mg/kg bw/d	The low water solubility of this substance may limit oral bioavailability. Data indicate rapid hydrolysis (<1 min) to form ethanol and polymeric reaction products, so any systemic absorption is likely to be due to ethanol	Low toxicity profile	100 mg/kg bw/d	Renal effects seen in the 90-day study at 300 and 1000 mg/kg bw/d in males. Reduced bodyweight gain and increased kidney weights were also seen at 1000 mg/kg bw/d. One mortality (associated with severe renal inflammation) was seen in a male at 300 mg/kg bw/d. Renal histopathology in this study was characterised by multifocal interstitial nephritis and scarring, multifocal dilated cortical tubules and an increase in basophilic tubules and mononuclear cell infiltrates. No effects of clear toxicological significance were observed in females.
432-070-7	1000 mg/kg bw/d	Some effects seen in the 28-day study at the limit dose of 1000 mg/kg bw/d, but these were not considered to be of toxicological significance and/or not clearly related to treatment	Low toxicity profile	250 mg/kg bw/d	Changes in haematology, clinical chemistry and histopathology of the kidneys, bladder and stomach seen in the 90-day study at 1000 mg/kg bw/d.
470-680-5	1000 mg/kg bw/d	Two 28-day studies; one with minor/adaptive effects at the highest dose level of 1000 mg/kg bw/d; a second study performed at 2000 mg/kg bw/d shows effects on some parameters but these are disputed by an independent reviewer (but in any case are above the limit dose).	UNSURE	–	A 90-day study was not performed: not required at the registered tonnage band
204-111-7	1000 mg/kg bw/d	Evidence of adaptive effects at the highest dose level of 1000 mg/kg bw/d in the 28-day study and renal effects in the male rat (only) are dismissed as non-relevant; justification for low toxicity would require a more detailed case for dismissing these effects.	UNSURE	15 mg/kg bw/d (M) 150 mg/kg bw/d (F)	Renal effects (tubular dilatation and nephropathy) seen in the 90-day study at 150 and 1000 mg/kg bw/d in males. Deaths of males were reported at dose levels of 150 (1/10) and 1000 mg/kg bw/d (3/10); findings were considered to be treatment-related; however no cause of death is reported
230-991-7	1000 mg/kg bw/d, [NOEL: 200 mg/kg bw/d]	Minor/reversible effects seen at the highest dose level of 1000 mg/kg bw/d in the 28-day study. NB substance is shown to cause developmental toxicity at dose levels not causing maternal toxicity	Low toxicity profile	100 mg/kg bw/d	Renal effects (urolithiasis and obstructive nephropathy) seen in the 90-day study at 300 and 1000 mg/kg bw/d in males.
203-326-3	1000 mg/kg bw/d [NOEL:300 mg/kg bw/d]	No effects of treatment at the limit dose of 1000 mg/kg bw/d in the 28-day study. OECD QSAR Toolbox predicts no bioavailability which would be consistent with the molecular weight and insolubility in water	Low toxicity profile	1000 mg/kg bw/d	No effects seen at the limit dose in the 90-day study
211-074-0	1000 mg/kg bw/d	Substance predicted to be bioavailable but rapidly metabolised and incorporated into normal metabolism	Low toxicity profile	400 mg/kg bw/d (M) 1000 mg/kg bw/d (F)	Minor bodyweight effects seen in males at 1000 mg/kg bw/d in the 90-day study. Considered to still meet profile.
203-838-7	1750 mg/kg bw/d	Substance is classified for acute inhalation toxicity (borderline) but this is likely to be related to its corrosivity. Toxicity was seen at the highest dose level of 3500 mg/kg bw/d in a 28-day study; effects are most likely related to local irritation caused by gavage. NOAEL of 1750 mg/kg bw/d exceeds the limit dose. Substance is expected to be absorbed and rapidly metabolised (fatty acid)	Low toxicity profile	1000 mg/kg bw/d	No effects seen at the limit dose in the 90-day study
426-040-2	1000 mg/kg bw/d	No effects at the limit dose; substance not predicted to be bioavailable based	Low toxicity profile	1000 mg/kg bw/d	No effects seen at the limit dose in the 90-day study

Table 1 (*continued*)

EC No.	28-day NOAEL (rat, oral) result	Comments on the 'low toxicity profile'	Prediction made in Taylor et al. (2014)	90-day NOAEL (rat, oral) result	Comments on the 90-day study result
		on the molecular weight and low water solubility			
641-136-6	1000 mg/kg bw/d	No effects at the limit dose of 1000 mg/kg bw/d in the 28-day study; the substance is likely to be of limited or no bioavailability based on its low water solubility	Low toxicity profile	1000 mg/kg bw/d	No effects seen at the limit dose in the 90-day study
271-237-7	1000 mg/kg bw/d (F) 300 mg/kg bw/d (M)	28-day reports a NOAEL of 300 mg/kg bw/d in males, apparently based on reduced weight gain. Other effects at the highest dose level are dismissed as adaptive or non-adverse. Substance is a UVCB and only a proportion of the components are likely to be bioavailable. However, the NOAEL in males cannot be disregarded.	UNSURE	1000 mg/kg bw/d	No effects seen at the limit dose in the 90-day study
271-239-8	1000 mg/kg bw/d (F) 300 mg/kg bw/d (M)	28-day NOAEL of 300 mg/kg bw/d for males based on a marginal effect on red blood cell parameters (increased erythrocyte count – not adverse?) at the highest dose level of 1000 mg/kg bw/d	Low toxicity profile	–	A 90-day study was not performed: not required at the registered tonnage band
404-370-8	1000 mg/kg bw/d	Effects at the limit dose of 1000 mg/kg bw/d in the 28-day study are minimal/adaptive. The intact substance is unlikely to be bioavailable, however systemic exposure to the hydrolysis product is likely (hydrolysis at pH 4 is very rapid)	Low toxicity profile	–	Read-across study provided; NOAEL = 1000 mg/kg bw/d
402-140-1	1000 mg/kg bw/	Effects at the limit dose of 1000 mg/kg bw/d in the 28-day study are minimal/adaptive. The intact substance is unlikely to be bioavailable, however systemic exposure to the hydrolysis product is likely (hydrolysis at pH 4 is very rapid)	Low toxicity profile	–	Read-across study provided; NOAEL = 1000 mg/kg bw/d

a 90-day study is requested, conduct of the 90-day study is dependent on the results of the 28-day study (another option is to request the 90-day study only). Consideration should be given as to whether it is necessary to conduct the 90-day study if there are no significant, potentially human-relevant, toxicological findings in the 28-day study. Confidence in the 'low (sub)acute toxicity profile' of a substance is further increased if there is no evidence of any toxicological activity (for example in genotoxicity, acute toxicity and sensitisation studies) and if the physical/chemical properties of the substance suggest that it is of low biological availability.

It should be noted that relatively few substances will fit this kind of chemical space; only approximately 10% of substances so far registered on the various databases appear to fit this profile. Nonetheless, any opportunity to avoid unnecessary animal testing should be taken where possible. There will be more substances for which there is now 90-day study data available (see for example, Luechtefeld et al., 2016), including more substances for which testing proposals have been submitted since our cut-off date of 1 September 2012, so it is possible to further test our hypothesis with more substances.

Acknowledgments

Author Taylor was supported by funding from the Cruelty Free International Charitable Trust during the drafting of this manuscript.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.yrtph.2017.09.018.

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