

Cobalt Institute

May 7th, 2019

PUBLIC COMMENT PERIOD FOR: - “Draft Hot Spots Cancer Inhalation Unit Risk Factors for Cobalt and Cobalt Compounds.”

PRELIMINARY COMMENTS ON: - “Technical Support Document for Cancer Potency Factors; Appendix B; Public Comment Draft; March 2019.”

TO THE OFFICE OF ENVIRONMENTAL HEALTH ASSESSMENT (California)

The Cobalt Institute (CI) is a global, non-profit trade association composed of producers, users, recyclers, and traders of cobalt (Co). We promote the sustainable and responsible production and use of cobalt in all its forms. The CI acts as a knowledge center for governments, agencies, industry, the media and the public on all matters concerning Co and Co containing substances. Our technical expertise includes Co related health, safety, and environmental issues.

We welcome the opportunity to comment on your above document deriving cancer slope factors for Co and Co compounds. Based on a thorough scientific review, we have already submitted preliminary comments in April 2019. These are herewith followed up and complemented with a more detailed response into the public consultation. We thank you for your attention to these comments.

EXECUTIVE SUMMARY

In response to the California OEHHA “Air Toxics Hot Spot Program”: Cobalt and Cobalt Compounds Cancer Inhalation Unit Risk Factors, **we are concerned that there are 4 separate very conservative assumptions. Their combination results in a “multiplication of conservatism” that in turn results in a significant overestimation of risk.** We would like to make comments related to the following points:

1 – Mutagenicity/genotoxicity of cobalt compounds:

Cobalt and cobalt compounds are not mutagenic, and do not display in vivo genotoxicity. The mode of action of cobalt related carcinogenicity is via reactive oxygen species, hypoxia and inflammation.

2 – Independence of tumors:

In both NTP cancer bioassays (cobalt sulfate and cobalt metal powder), adrenal pheochromocytoma were observed. These tumors are a well-known secondary response to respiratory distress of any origin (not just lung cancer) and should not be interpreted as independent cobalt-related tumors. Further systemic tumors were only seen in one sex of rats in the cobalt metal powder study (not in mice). The rat strain and colony used in this particular inhalation study does not have a historical control database against which these tumors can be compared, making the interpretation of these findings extremely difficult. Further, there was no exposure-response in any of these findings. The conclusion of “independence” of these tumors appears therefore premature.

3 – Solubility of Co metal powder:

While cobalt metal powder is moderately soluble in water, it is in fact highly soluble in biologically relevant fluids, including all lung fluids, and releases similar and higher amounts of cobalt ion as a soluble cobalt salt. Cobalt metal powder is a member of the group of bioavailable cobalt compounds and cannot be taken as a representative of poorly soluble cobalt compounds.

4 – BMD05 calculations with “high effects” data:

BMD05 calculations with the data from the cobalt metal study alone result in an extremely low BMDL05.

This reflects the high uncertainty related to the modeling and is not an indication that Co metal powder is a more potent carcinogen than Co sulfate. Modeling the Co sulfate and the Co metal powder data in one dose-response is presented, demonstrating that the responses are strictly exposure concentration dependent, and not a result of different potencies.

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DETAILED COMMENTS

1 – In vivo genotoxicity of Co metal and Co compounds (referred to as “Co compounds” in the below comments)

The assumption of in vivo genotoxicity of Co compounds is based on data from studies with a low “Klimisch score”, mainly based on non-relevant route of exposure (intra-peritoneal injection), low reliability based on flaws in reporting, and the fact that these studies did not follow OECD guidelines for genotoxicity testing.

We would like to highlight to OEHA an OECD review of 2014

(<https://hpvchemicals.oecd.org/ui/handler.axd?id=e5e60085-1f3f-4df5-92f6-8f32c26c3082>) which concludes lack of in vivo genotoxicity of Co compounds, following a stringent quality, reliability and relevance screening of the genotoxicity database of Co compounds. This conclusion is also reflected in recent publications [1, 2].

Further work has very recently been conducted by the CI and Cobalt EU REACH Consortia (CoRC), using a novel assay specifically developed to distinguish between genotoxic versus non-genotoxic carcinogens. The assay is called “ToxTracker” and is a panel of mammalian stem cell lines (mouse embryonic stem cells) that contain different fluorescent reporters representing four distinct biological responses that are associated with carcinogenesis, i.e. general cellular stress, DNA damage, oxidative stress and the unfolded protein response [3]. The differential induction of the Green Fluorescent Protein (GFP) reporters as well as cytotoxicity of the tested compounds were determined by flow cytometry. Upregulation of hypoxia genetic markers was determined by quantitative Polymerase Chain Reaction (qPCR). Co metal powder and the highly soluble and bioavailable Co salt CoCl_2 -hexahydrate were tested in this system. The results confirm the previous conclusions that Co compounds do not induce DNA damage, and instead are potent inducers of oxidative stress and hypoxia.

The ToxTracker data will be incorporated into an Adverse Outcome Pathway hypothesis for bioavailable Co compounds, and will be published before end of 2019. The ToxTracker method is currently undergoing OECD and ECVAM review and evaluation to become an OECD guideline method for testing of genotoxic versus non-genotoxic chemicals.

2 - Assumption of “independence” of tumors in Co inhalation studies

There were exposure-concentration dependent increases in the incidences of benign and malignant pheochromocytoma (combined) in all substance-exposed male and female rats. This effect was not observed in mice. These tumors are well-established responses that are secondary to hypoxia and respiratory distress (adrenal pheochromocytoma in rats [4]).

In a statistical re-evaluation of nine, 2-year NTP inhalation studies, a range of lung effects (chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, squamous metaplasia, proteinosis, and histiocytosis) and their association with pheochromocytoma was investigated. It was concluded that there is an overall association between lung impairment by any cause and an elevated incidence of adrenal pheochromocytoma in NTP inhalation studies. The elevated incidences of pheochromocytoma in rats after inhalation exposure to Co metal are considered to be rat-specific responses to respiratory distress, with no causal relationship to Co. Also, there is no indication for an involvement of genotoxic mechanisms in the induction of pheochromocytoma by chemicals in animals [4, 5].

Therefore, these tumors should not be assumed to be occurring independently, as this is not supported by the MoA leading to pheochromocytoma in inhalation studies and may lead to a severe overestimation of the potency of Co ion related carcinogenicity.

The assumption of independence of the tumors warrants a closer look at all tumorigenic findings in the NTP inhalation studies with Co sulfate and Co metal powder:

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Rare systemic tumors in the context of historical control data

Historical control data are needed to decide whether a tumor is “rare” (background rate of < 1%) or “common” (background rate > 1%) and are needed to interpret the significance especially of rare tumors and of marginally increased tumor incidences. In the NTP Co metal inhalation study, the tumors in kidney and pancreas can probably be considered “rare”, however, in this context, it needs to be outlined that there are no historical control data for the F344 NTac strain (the F344N colony at Taconic laboratories) and inhalation exposure route (in that strain) at NTP. In total, only two carcinogenicity studies were carried out at NTP with the F344 NTac rats, one by inhalation (the Co metal study) and one by p.o. route of exposure (TR 583, Bromodichloroacetic Acid, drinking water study). The “historical control” used by the NTP in the Co metal report consisted of only 100 animals, which actually includes the concurrent control (50 animals), with the addition of another 50 animals of study TR 583, exposed by a different route of exposure. This is not what would constitute a “historical control”. For comparison, a typical historical control database would consist of around 50 studies by the same route of exposure, and several thousand animals [6].

Why are there no historical control data for the rat colony F344NTac used in the Co metal inhalation study?

Only one inhalation carcinogenicity study was ever conducted at the NTP with the F344NTac rat. It is important to realize that the F344NTac rats had developed a number of problems specific to this colony, including “declining fertility, sporadic seizure activity, and chylothorax” [7].

A specialty group set-up by the NTP (“rat breakout group”) notes that these issues “have occurred within the past 5 years in the NTP F344/N rat colony.” The NTP Co metal inhalation study range finders were finalized in 2005, meaning that the study design for the chronic study, including selection of rat strain and colony were already decided and underway by the time this report was issued. The report continues that “These issues are unique to our F344/N colony maintained at Taconic Farms, Inc. and to the best of our knowledge do not appear in other colonies maintained for commercial purposes at Taconic or other suppliers. The reasons for the development of these conditions in this specific colony have not been identified”. This led to the strong recommendation of the expert group to discontinue the use of this rat strain and colony, which was implemented by the NTP immediately.

Due to the increasing morbidity of the F344/NTac colony and the lack of historical control data, the occurrence of the systemic tumors in the Co metal study cannot be conclusively interpreted.

Common systemic tumors: Mononuclear cell leukemia (MNCL)

While there was an increase in MNCL at all exposure levels in female rats, the increase was not exposure level-related (incidence was highest at the lowest exposure level). In addition, there was no significant increase of MNCL in male rats. This finding did not occur in mice.

MNCL occurs with a high spontaneous background rate, and occurred at 42% and 36% in the controls, males and females, respectively. The incidence of MNCL is high across all exposure groups in the male rats, including controls (42%, 50%, 44%, 44% in control, 1.25, 2.5 and 5 mg Co/m³ exposure groups, respectively); it is also high in all female rats with 36%, 62%, 61%, 59% in control, 1.25, 2.5 and 5 mg Co/m³ exposure groups, respectively. The female control animals display an in fact somewhat low incidence of MNCL. These data reflect the general observation that MNCL is a common tumor type, and that Fisher rats are generally prone to developing MNCL as they age [8]. Extremely elevated incidences of MNCL have been previously observed in a number of chronic bioassays and 2-year carcinogenicity studies in F344 rats [9, 10]. The analysis of the spontaneous neoplasm incidences in F344 rats from chamber controls of 18 two-year inhalation studies carried out by the NTP revealed a frequent occurrence of MNCL in males (57.5%, range 34-70%) and in females (37.3%, range 24-54%) [9]. The data show that MNCL occurs in untreated aged rats

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at extremely high and variable rates. The conclusion that MNCL is a Co related tumor based on the data in female rats cannot be substantiated when taking into account the data from both sexes, and when taking into account the high and variable occurrence of this common tumor.

MNCL is uncommon in most other rat strains, and its background incidence in the Fisher rat has increased significantly over time. MNCL has not been found in other mammalian species and no histologically comparable tumor is found in humans [10]. In the light of the well-known occurrence of MNCL in the Fisher rat, this result does not suggest that this is an independently occurring tumor directly related to Co exposure.

Kidney, adenoma/carcinoma combined

There was a minimal increase in the incidence of these tumors in male rats, although not statistically significant. Because of this slight increase an extended review using “step-sections” was conducted. Using these extended data there is no evidence of a carcinogenic response in male rats, which is supported by the lack of an increase in tubular hyperplastic changes or in kidney tumors in female rats or in male and female mice.

The neoplasms in the kidney were slightly above the concurrent control data, but not statistically significant and no overall positive trend was established. In the light of these arguments, these findings do not appear to warrant an assumption that these tumors are independently occurring and related to Co exposure.

Pancreatic islets

There was a small increase in islet-cell tumors in the mid- and high-dose male rats but not in female rats (a small but not statistically non-significant increase was seen in the highest dose group). Mice did not display this effect.

These tumors are rare, and they were seen for the first time in an NTP study. Also, the F344 NTac rat was used for the first, and only, time in an NTP inhalation study. It is impossible to interpret these findings, and the statement in the NTP report that there was “equivocal evidence of carcinogenic activity” is considered justified. This level of evidence should not be taken as a basis for a conclusion that these are independently occurring tumors caused by exposure to Co.

Apart from the pheochromocytoma, systemic tumors were observed exclusively in the inhalation study with Co metal powder. This may be related to the very high exposure concentrations (adjusted for Co equivalent, the lowest dose in the Co powder study was higher than the highest dose in the Co sulfate study), or it may reflect the health issues that have led to the immediate discontinuation of the use of the F344NTac colony in NTP cancer bioassays.

In summary, several aspects cast doubt on the interpretation that the individual systemic tumors are independent and directly related to Co:

- The predominant finding (adrenal pheochromocytoma) is a well-known response to respiratory distress and hypoxia
- For the remaining systemic tumors, the following points can be made:
 - There is a lack of an exposure-response relationship
 - They occurred only in one sex (either males or females) of the rats
 - There is a complete lack of a historical control database for this rat colony (F344NTac), making it impossible to conclude whether the systemic tumors are biologically relevant or statistically significant

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- This rat colony is uniquely sensitive and had developed a number of spontaneous diseases that immediately (after one inhalation study) led to the discontinuation of the use of this colony at NTP

3 – Assumption of low solubility of Co metal powder

While Co metal powder is poorly soluble in water, it is in fact moderately to highly soluble in biological fluids, such as interstitial, alveolar or lysosomal artificial lung fluids. Data on the bioelution of several Co compounds in lung fluid has led to the grouping of Co metal powder with the “soluble salts” (Co sulfate, Co chloride, Co nitrate and Co acetate) in one group of Co compounds classified as inhalation carcinogens (Carc 1B). This group of compounds is characterized by the induction of an inflammatory response and hypoxia in the lung following inhalation exposure. The similarity in effects caused by this group of substances has led to the conclusion that the toxicity of Co compounds is related to the Co ion, and that the magnitude of effect is related to the Co ion dose-to-target. This also inherently assumes that dose-to-target is critical for the magnitude of effect, and not differences in the potency between Co substances. This assumption is confirmed by the evaluation of the dose-response of Co exposure (from Co sulfate and Co metal powder) across all exposure concentrations in both NTP studies. The combination of both Co compounds into one dose response curve results in very good model fit, and the indication that the model is able to predict exposure-responses at relevant (low) exposures. A detailed report on benchmark dose (BMD) modeling of the complete animal dataset (Co metal powder and Co sulfate) is appended to these comments.

It is important to note that there are substances with negligible solubility in biological fluids (e.g., Co_3O_4 and CoS). Bioelution data exist indicating that these “biologically insoluble” substances should not be grouped with Co metal powder for the endpoint inhalation toxicity. These bioelution data are currently being written up into a manuscript for publication (together with the mechanistic data generated by the ToxTracker assay mentioned earlier). CI is willing to share / discuss bioelution, but not to put data in the public domain before publication.

4 - Calculation of BMDL5 with Co metal data only

A serious concern arises related to the use of the BMD model in the context of the Co metal data alone. Doses/exposures are needed that produce different effect sizes providing information on both the lower and higher part of the dose–response relationship to characterize the full dose–response relationship [11]. Limitations in data can arise from a relatively high response at the lowest dose [11], and it can be concluded that using more but smaller dose groups definitely does not deteriorate BMD precision, but rather may have a positive impact on the performance of the study [12]. Indeed, it has been suggested that the magnitude of uncertainty of the BMD estimate, as indicated by the BMDL–BMDU ratio, should be used as a tool for evaluating the statistical quality of the underlying data [13], and the utility of a BMDL as a reference PoD for regulatory decision-making [13-15].

In the Co metal powder study, at the lowest dose, 30% of the female rats and 50% of the male rats had lung tumors. Extrapolation from high dose/high response data into areas of lower responses (e.g. BMD10 or 05) that are this far outside the data results in high uncertainty and very large differences between the BMDL-BMDU ratio (BMD upper and lower confidence limits).

A BMDL05 calculation based on Co metal data (male rats) alone shows that the ratio between BMDL and BMDU at 5% risk is 24, demonstrating the high uncertainty of the modeled BMD05 values. This uncertainty is significantly reduced, with a BMDL-BMDU ratio of 3.75, when the Co sulfate data are included in the dose response modeling. The reduction in the uncertainty is a result of the Co sulfate exposures, which were all lower than those applied in the Co metal study when compared on the Co equivalent basis. The BMD5 modeling using all data (Co sulfate and Co metal powder), both rats and mice, males and females, reduces

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the BMDL-BMDU ratio to 3. There appears to be a good dose-response fit across all studies (Co metal powder and Co sulfate, rats-mice, male-female), rather than an elevated potency of Co metal powder versus Co sulfate. This indicates that the responses are related to the Co equivalent exposure concentration, and not to a difference in potency between Co metal powder and Co sulfate.

In closing, the CI trusts our comments provided above are helpful to provide a better understanding of the reasons for our concerns. We would be pleased to discuss any questions you may have.

Yours,



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APPENDIX – BMD modelling reports

A - Co metal powder data alone, male rats

B - All data (Co sulfate and Co metal powder), male rats

(unformatted BMD reports following the EFSA template are provided, to show the data entered, the resulting BMDL and BMDU, as well as the visualization of the curve fits)

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Benchmark Dose Modeling: Report

European Food Safety Authority (EFSA)

Acknowledgements: [Scientific Committee OR EFSA] wishes to thank the following for the support provided to this scientific output: [staff members or others who made a contribution but are not eligible as authors]. The Panel [Scientific Committee OR EFSA] wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Individual authors [add names in the format Surname followed by Initial(s), Surname followed by Initial(s) and Surname followed by Initial(s)], 20YY. Title of the report. EFSA supporting publication 20YY:EN-NNNN. 10 pp. doi:10.2903/sp.efsa.20YY.EN-NNNN

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Summary

BMD modeling of the cobalt metal powder inhalation data in male rats.

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Data Description

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The endpoint to be analyzed is: tumors.

Data used for analysis:

dose	tumors	N
0.00	2	50
1.25	25	50
2.50	39	50
5.00	44	50

Information pertaining to this endpoint.

Selection of the BMR

The BMR (benchmark response) used is an extra risk of 5% compared to the controls.

When the specified BMR deviates from the default value, the rationale behind the choice made should be described.

The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 95% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 66.27, for the underlying calculations.

Specification of Deviations from Default Assumptions

General assumptions

Please motivate in detail assumptions made when deviating from the recommended defaults (e.g. gamma distributional assumption instead of log-normal, heteroscedasticity instead of homoscedasticity).

Dose-response models

Other models than the recommended ones that were fitted should be listed, with the respective description of reasons to include them.

Default set of fitted models:

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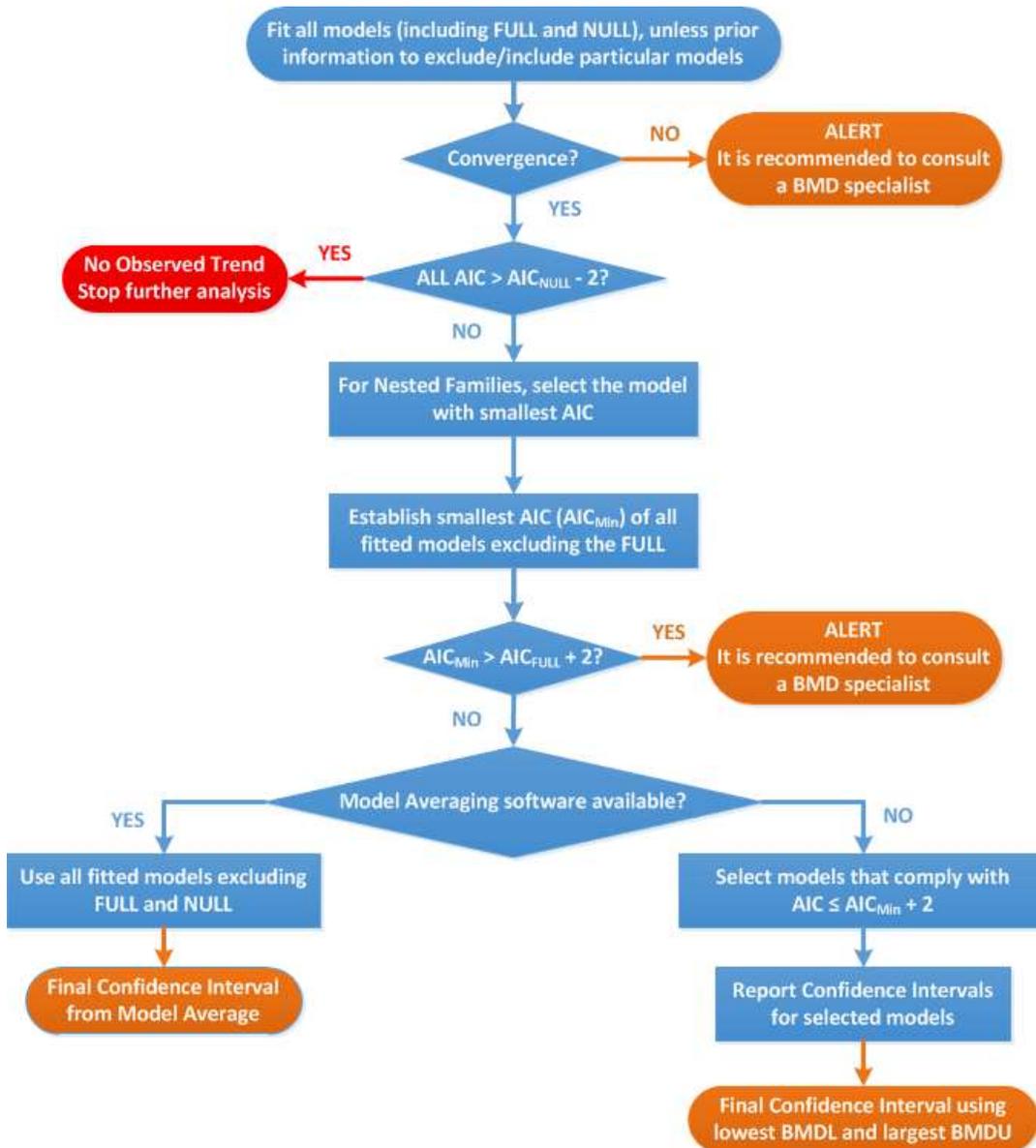
Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{group mean}$
Logistic	2	$y = \frac{1}{1 + \exp(-a - bx)}$
Probit	2	$y = \text{pnorm}((x - a) \cdot b)$
Log-logistic	3	$y = a + \frac{1 - a}{1 + \exp\left(c \cdot \log\left(\frac{b}{x}\right)\right)}$
Log-probit	3	$y = a + (1 - a) \cdot \text{pnorm}\left(c \cdot \log\left(\frac{x}{b}\right)\right)$
Weibull	3	$y = a + (1 - a) \left(1 - \exp\left(-\left(\frac{x}{b}\right)^c\right)\right)$
Gamma	3	$y = \text{pgamma}(bx; c)$
Two-stage	3	$y = a + (1 - a) \left(1 - \exp\left(-\frac{x}{b} - c\left(\frac{x}{b}\right)^2\right)\right)$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 5	4	$y = a \cdot (c - (c - 1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 5	4	$y = a \cdot \left(1 + (c - 1) \frac{x^d}{b^d + x^d}\right)$

For the Exp and Hill family, we fit models with 3 and 4 parameters as listed in the table. The 3-parameter model is selected if the difference in AIC is smaller than 5, otherwise the 4-parameter model is selected.

Procedure for selection of BMDL

Description of any deviation from the procedure described in the flow chart to obtain the final BMD confidence interval.

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Flowchart for selection of BMDL

Results

Response variable: tumors

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
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null	1	-137.63	277.26		NA	NA	NA	NA
full	4	-87.75	183.50		NA	NA	NA	NA
two.stage	3	-88.67	183.34	yes	8.25e-02	0.130	0.1030	yes
log.logist	3	-87.96	181.92	yes	2.01e-02	0.429	0.1840	yes
Weibull	3	-88.26	182.52	yes	1.57e-03	0.191	0.0496	yes
log.prob	3	-88.04	182.08	yes	2.33e-02	0.450	0.1970	yes
gamma	3	-88.31	182.62	yes	6.86e-05	0.238	0.0368	yes
logistic	2	-96.57	197.14	no	NA	NA	0.2900	yes
probit	2	-97.62	199.24	no	NA	NA	0.2870	yes
LVM: Expon. m3-	3	-88.31	182.62	yes	7.03e-04	0.095	0.0173	yes
LVM: Hill m3-	3	-88.23	182.46	yes	8.19e-04	0.150	0.0353	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.04246

estimate for BMD- : 0.1028

estimate for c : 1e-06

log.logist

estimate for a- : 0.03974

estimate for BMD- : 0.1838

estimate for c : 1.527

Weibull

estimate for a- : 0.03955

estimate for BMD- : 0.04961

estimate for c : 0.8184

log.prob

estimate for a- : 0.03974

estimate for BMD- : 0.1973

estimate for c : 0.8914

gamma

estimate for a- : 0.03968

estimate for BMD- : 0.03683

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estimate for cc : 0.707

logistic

estimate for a- : -1.595

estimate for BMD- : 0.2897

probit

estimate for a- : -0.9259

estimate for BMD- : 0.2868

EXP

estimate for a- : 1.555

estimate for CED- : 0.01727

estimate for d- : 0.3555

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.554

estimate for CED- : 0.03531

estimate for d- : 0.478

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

Weights for Model Averaging

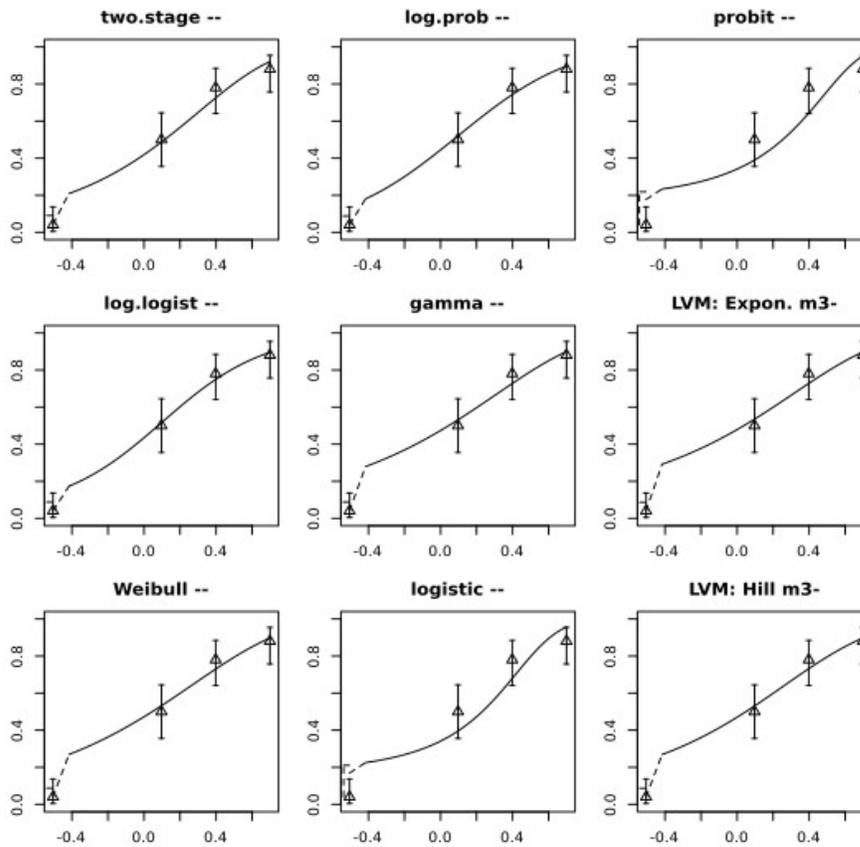
two.stage	log.logist	Weibull	log.prob	gamma	logistic	probit	EXP	HILL
0.09	0.19	0.14	0.17	0.13	0	0	0.13	0.14

Final BMD Values

subgroup	BMDL	BMDU
	0.01	0.24

Confidence intervals for the BMD are based on 200 bootstrap data sets.

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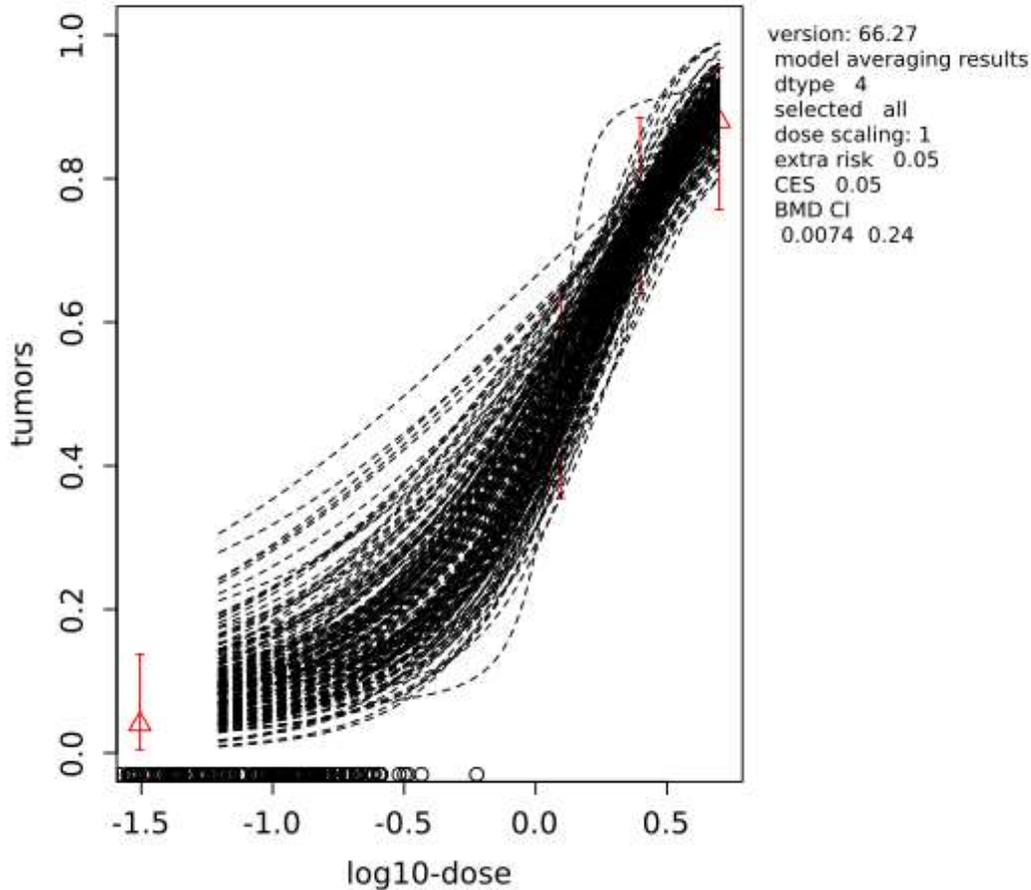
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bootstrap curves based on model averaging



Conclusions

Using cobalt metal powder data alone results in high uncertainties of the model at the lower end of the dose response curve.

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Benchmark Dose Modeling: Report

European Food Safety Authority (EFSA)

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Summary

BMD05 modeling of cobalt sulfate hexahydrate and cobalt metal powder inhalation data in one dose response curve.

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Summary

1. Data Description
2. Selection of the BMR
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Data Description

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The endpoint to be analyzed is: tumors.

Data used for analysis:

dose	tumors	N
0.000	1.5	50
0.067	4.0	50
0.224	4.0	48
0.672	7.0	50
1.250	25.0	50
2.500	39.0	50
5.000	44.0	50

Information pertaining to this endpoint.

Selection of the BMR

The BMR (benchmark response) used is an extra risk of 5% compared to the controls.

When the specified BMR deviates from the default value, the rationale behind the choice made should be described.

The BMD (benchmark dose) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software Used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 66.20, for the underlying calculations.

Specification of Deviations from Default Assumptions

General assumptions

Please motivate in detail assumptions made when deviating from the recommended defaults (e.g. gamma distributional assumption instead of log-normal, heteroscedasticity instead of homoscedasticity).

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Dose-response models

Other models than the recommended ones that were fitted should be listed, with the respective description of reasons to include them.

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	no. of groups	$y = \text{group mean}$
Logistic	2	$y = \frac{1}{1 + \exp(-a - bx)}$
Probit	2	$y = \text{pnorm}((x - a) \cdot b)$
Log-logistic	3	$y = a + \frac{1 - a}{1 + \exp\left(c \cdot \log\left(\frac{b}{x}\right)\right)}$
Log-probit	3	$y = a + (1 - a) \cdot \text{pnorm}\left(c \cdot \log\left(\frac{x}{b}\right)\right)$
Weibull	3	$y = a + (1 - a) \left(1 - \exp\left(-\left(\frac{x}{b}\right)^c\right)\right)$
Gamma	3	$y = \text{pgamma}(bx; c)$
Two-stage	3	$y = a + (1 - a) \left(1 - \exp\left(-\frac{x}{b} - c \left(\frac{x}{b}\right)^2\right)\right)$
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 5	4	$y = a \cdot (c - (c - 1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$
Hill model 5	4	$y = a \cdot \left(1 + (c - 1) \frac{x^d}{b^d + x^d}\right)$

For the Exp and Hill family, we fit models with 3 and 4 parameters as listed in the table. The 3-parameter model is selected if the difference in AIC is smaller than 5, otherwise the 4-parameter model is selected.

Procedure for selection of BMDL

Description of any deviation from the procedure described in the flow chart to obtain the final BMD confidence interval.

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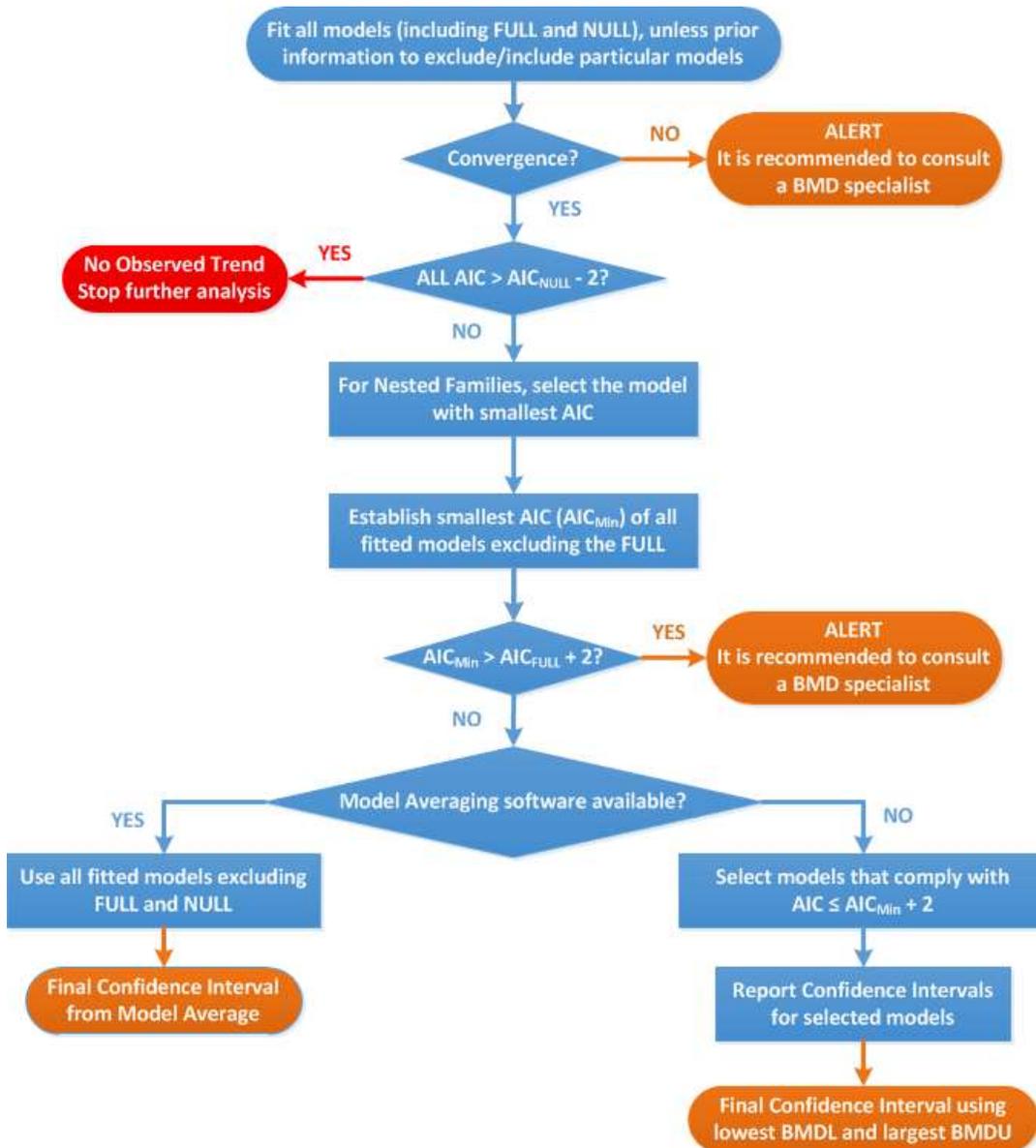
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Flowchart for selection of BMDL

Results

Response variable: tumors

Fitted Models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	conv
-------	--------	--------	-----	----------	------	------	-----	------

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null	1	-226.94	455.88		NA	NA	NA	NA
full	7	-134.04	282.08		NA	NA	NA	NA
two.stage	3	-138.89	283.78	no	NA	NA	0.1210	yes
log.logist	3	-136.49	278.98	yes	0.199	0.497	0.3370	yes
Weibull	3	-138.46	282.92	no	NA	NA	0.1660	yes
log.prob	3	-136.84	279.68	yes	0.232	0.535	0.3740	yes
gamma	3	-138.22	282.44	no	NA	NA	0.2050	yes
logistic	2	-146.56	297.12	no	NA	NA	0.3640	yes
probit	2	-147.92	299.84	no	NA	NA	0.3540	yes
LVM: Expon. m3-	3	-138.86	283.72	no	NA	NA	0.0955	yes
LVM: Hill m3-	3	-138.20	282.40	no	NA	NA	0.1330	yes

Estimated Model Parameters

two.stage

estimate for a- : 0.02824

estimate for BMD- : 0.1214

estimate for c : 0.06571

log.logist

estimate for a- : 0.05166

estimate for BMD- : 0.3369

estimate for c : 1.965

Weibull

estimate for a- : 0.03529

estimate for BMD- : 0.1661

estimate for c : 1.145

log.prob

estimate for a- : 0.05609

estimate for BMD- : 0.3745

estimate for c : 1.163

gamma

estimate for a- : 0.04058

estimate for BMD- : 0.2048

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estimate for cc : 1.344

logistic

estimate for a- : -2.175

estimate for BMD- : 0.3636

probit

estimate for a- : -1.262

estimate for BMD- : 0.3541

EXP

estimate for a- : 1.659

estimate for CED- : 0.09553

estimate for d- : 0.4645

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

HILL

estimate for a- : 1.621

estimate for CED- : 0.1329

estimate for d- : 0.6349

estimate for th(fixed) : 0

estimate for sigma(fixed) : 0.25

Weights for Model Averaging

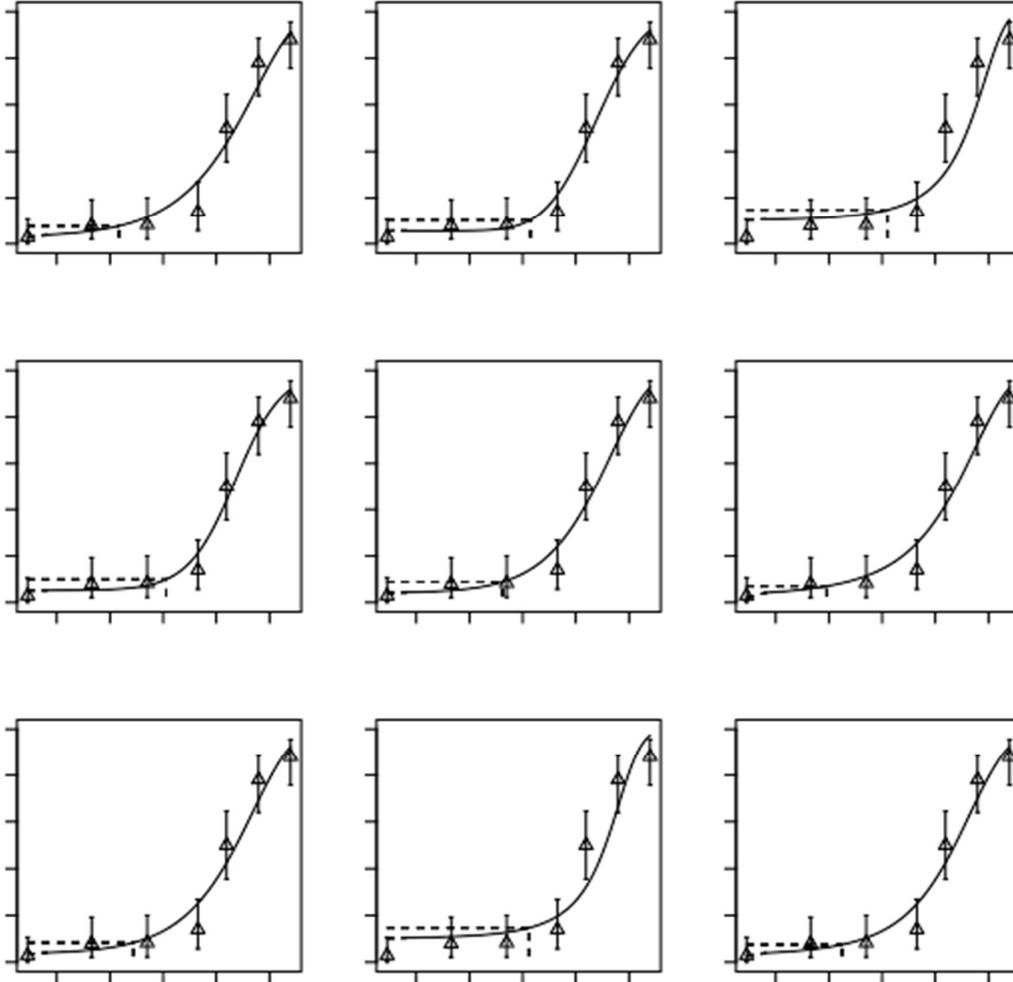
two.stage	log.logist	Weibull	log.prob	gamma	logistic	probit	EXP	HILL
0.04	0.42	0.06	0.3	0.07	0	0	0.04	0.08

Final BMD Values

BMDL	BMDU
0.12	0.45

Confidence intervals for the BMD are based on 200 bootstrap data sets.

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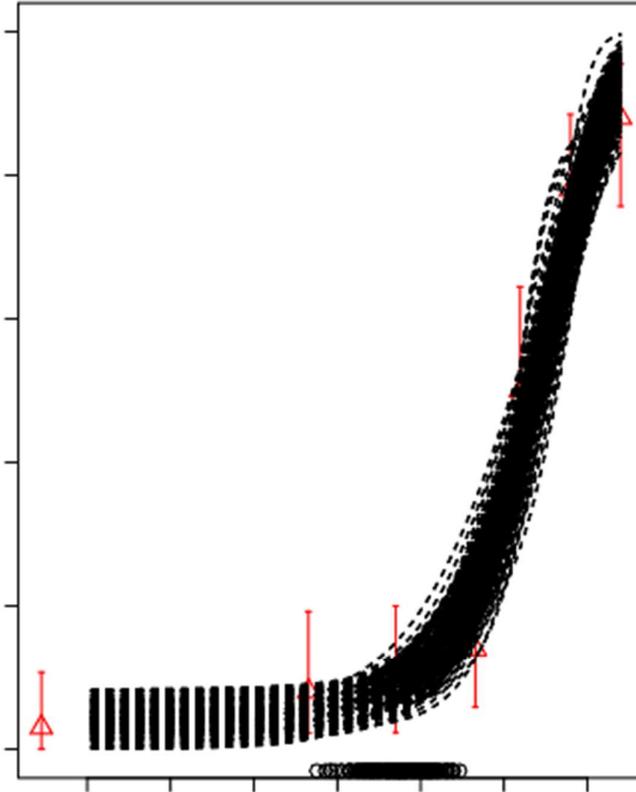
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Conclusions

The dose response of the two inhalation studies combined (cobalt sulfate hexahydrate and cobalt metal powder NTP inhalation studies) results in good model fits and in reduced uncertainty in the lower end of the dose response curve.