Le directeur général

Maisons-Alfort, le 11 mars 2015

AVIS
de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

relatif à « la demande d'actualisation des résultats de la deuxième étude de l'alimentation totale (EAT2) relatifs à l'exposition des consommateurs aux toxines T2 et HT2 à la lumière de la nouvelle valeur toxicologique de référence établie par l'EFSA »

L'Anses a été saisie le 7 février 2012 par la Direction générale de la concurrence, de la consommation et de la répression des fraudes (DGCCRF) d'une demande d'avis relatif à l'actualisation des résultats de la deuxième étude de l'alimentation totale (EAT2) concernant l'évaluation de risque lié à l'exposition des consommateurs aux toxines T2 et HT2 à la lumière de la nouvelle valeur toxicologique de référence établie par l'EFSA.

CONTEXTE ET OBJET DE LA SAISINE

L'EFSA a adopté, le 30 novembre 2011, un avis sur les risques, pour la santé humaine et pour la santé animale, liés à la présence des toxines T2 et HT2 dans les denrées alimentaires et dans les aliments pour animaux. Une dose journalière tolérable (DJT) de 100 ng $kg.pc^{-1}$ a été établie pour la somme de ces deux toxines. L'EFSA a conclu à l'absence de préoccupation sanitaire pour ces mycotoxines car l'exposition chronique estimée de toutes les catégories de consommateurs prises en compte est inférieure à la valeur toxicologique de référence nouvellement établie :

- Pour les adultes, l'exposition moyenne est comprise entre 3,4 et 18 ng $kg.pc^{-1}$ j^{-1}. Au 95ème percentile, elle est comprise entre 7,2 et 39 ng $kg.pc^{-1}$ j^{-1}.
- Pour les enfants de 1 à 3 ans, l'exposition moyenne est comprise entre 12 et 43 ng $kg.pc^{-1}$ j^{-1}. Au 95ème percentile, elle est comprise entre 23 et 91 ng $kg.pc^{-1}$ j^{-1}.

Les résultats de la deuxième étude de l'alimentation totale (EAT2) relatifs à l'exposition des consommateurs français aux toxines T2 et HT2 se réfèrent à la dose journalière tolérable provisoire préalablement établie à 60 ng $kg.pc^{-1}$. Ces résultats indiquent que :

- Sous l'hypothèse basse, 0,1% des enfants ont une exposition supérieure à la valeur toxicologique de référence (non significatif) mais aucun adulte ne dépasse cette même valeur ;
- Sous l'hypothèse haute, 30% des adultes et 74% des enfants ont des expositions supérieures à la valeur toxicologique de référence.

L'Anses a estimé que le risque théorique ne peut être écarté pour ces mycotoxines et a souligné la nécessité d'abaisser les limites de quantification analytique au moins pour les contributeurs majeurs identifiés.

Suite à l'avis EFSA du 30 novembre 2011, la Commission européenne a initié des discussions sur les mesures de gestion à mettre en œuvre pour limiter le niveau de contamination en T-2 HT-2, notamment dans les produits céréaliers. Ainsi, le règlement 2013/165/UE du 27 mars 2013 recommande aux états membres, avec l'appui des opérateurs industriels, de mettre en place une surveillance visant à détecter la présence des toxines T-2 HT-2 dans les céréales et les produits à base de céréales ainsi que, en cas de dépassement des valeurs indicatives proposées, des enquêtes pour mieux comprendre l'origine des contaminations.

Dans le cadre de ces discussions au niveau communautaire, les autorités françaises souhaitent disposer d'une actualisation des résultats de l'EAT2 sur la base de la nouvelle valeur toxicologique de référence établie par l'EFSA.

ORGANISATION DE L'EXPERTISE

L'expertise collective a été réalisée par le comité d'experts spécialisés (CES) « Résidus et contaminants chimiques et physiques » (CES RCCP) devenu CES « Evaluation des risques physiques et chimiques dans les aliments» (CES ERCA) pendant l'instruction de cette saisine.
Ces travaux d'expertise ont été réalisés dans le respect de la norme NF X 50-110 « qualité en expertise ».

La présente expertise s'appuie sur un rapport initial rédigé par un expert du CES ERCA. Elle se base sur les données issues des avis de l'agence européenne de sécurité des aliments (EFSA, 2011), du Comité d'experts FAO/OMS sur les additifs alimentaires (JECFA, 2001) et du comité scientifique de la commission européenne en charge des questions alimentaires (SCF, 2001 et 2002) sur le sujet des mycotoxines T-2 et HT-2 ainsi que sur les études toxicologiques de Rafai *et al.* (1995) et de Meissonnier *et al.* (2008) et apporte des éléments d'éclairage sur la méthode employée par l'EFSA pour construire la DJT de 100 ng $kg.pc^{-1}\ j^{-1}$.

Le CES ERCA a adopté les travaux d'expertise collective ainsi que ses conclusions et recommandations, objets du présent avis, lors de sa séance du 17 mai 2013. Sur la base de cet avis, un courrier a été adressé à l'EFSA le 27 août 2014 pour signaler la position divergente du CES ERCA quant à la construction de la nouvelle valeur toxicologique de référence. Le CES ERCA a de nouveau été consulté le 14 octobre 2014 pour se prononcer sur les arguments avancés par l'EFSA en réponse au courrier du 27 août.

ANALYSE ET CONCLUSIONS DU CES ERCA

Suite à l'analyse de la méthode utilisée par l'EFSA pour la construction de la DJT de 100 ng $kg.pc^{-1}\ j^{-1}$, le CES ERCA est en désaccord avec l'approche retenue et considère plus pertinent d'établir son évaluation de risque sur la base de la DJMTP de 60 ng $kg.pc^{-1}$

préalablement utilisée pour l'analyse des résultats de l'EAT2. L'argumentaire développé par le CES a été transmis à l'EFSA (cf. annexe) le 27 août 2014.

Par ailleurs, le CES ERCA souligne qu'une comparaison entre les estimations des expositions de l'EFSA et de l'Anses est délicate compte-tenu des différences méthodologiques observées (taux de censure et hypothèses de gestion de la censure, performances des techniques analytiques, échantillons composites) sur le jeu de données initial.

L'EFSA, en date du 8 octobre 2014, a confirmé avoir suivi le document guide rédigé par son conseil scientifique et relatif à la méthodologie d'utilisation de l'approche « Benchmark Dose » à des fins d'évaluation du risque et a donc confirmé la valeur de 100 ng $kg.pc^{-1}$ j^{-1} pour les mycotoxines T-2 et HT-2. Néanmoins, l'EFSA a rappelé que cette méthodologie sera revue (notamment les critères d'acceptation ou de rejet des modélisations réalisées) en prenant en compte l'expérience acquise ces dernières années dans l'établissement de valeurs de référence.

Au regard des échanges ayant eu lieu entre les comités d'experts des 2 agences, le CES ERCA confirme que la dose journalière tolérable provisoire préalablement fixée à 60 ng $kg.pc^{-1}$ est plus robuste que celle retenue par l'EFSA et considère, par conséquent, qu'il n'est pas nécessaire d'actualiser les résultats de l'EAT2 relatifs à l'évaluation de risque lié à l'exposition des consommateurs aux toxines T-2 et HT-2.

CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail endosse les conclusions et recommandations du CES ERCA.

Le directeur général

Marc Mortureux

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MOTS CLES

Mycotoxines, toxine T2, trichothécènes, DJT, EFSA

ANNEXES

COLLECTIVE EXPERT APPRAISAL:

SUMMARY AND CONCLUSIONS

on the method used to develop the new toxicity reference value, set by EFSA on 30 November 2011, for T-2 and HT-2 mycotoxins

This document summarises the work of the Expert Committee on Assessment of the physical and chemical risks in foods

Scientific background to the expert appraisal

T-2 and HT-2 mycotoxins belong to the family of trichothecenes. They are produced by various *Fusarium* species. Those species grow on cereals (wheat, maize, rice, barley, etc.) and dried fruits, either directly in the field or during harvesting and storage, in regions with a temperate climate.
T-2 and HT-2 mycotoxins inhibit protein and DNA synthesis. They mainly cause immunosuppressive, but also haematotoxic and myelotoxic effects. They affect development and reproduction, as well as the nervous system at higher doses. The T-2 toxin is considered by the International Agency for Research on Cancer (IARC) as not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1993b).

In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) proposed a provisional maximum tolerable daily intake (PMTDI) of 60 ng $kg.bw^{-1}$ d^{-1} for the T-2 toxin and the HT-2 toxin, either alone or combined, on the basis of a lowest observed adverse effect level (LOAEL) of 29 µg $kg.bw^{-1}$ d^{-1} in piglets (Rafai, 1995) and a safety factor of 500. The critical effect selected is the toxicity on the immune system shown by a decrease, on the 21st day of exposure to T-2 mycotoxins (via feed), in the production of specific antibodies following immunisation with an extract of horse globulin (titre of anti-horse globulin antibodies) (JECFA, 2001b). This value was confirmed by the Scientific Committee on Food (SCF, 2001).

The results from the second total diet study (TDS2, ANSES, June 2011) relating to the exposure of French consumers to T-2 and HT-2 toxins refer to the PMTDI of 60 ng $kg.bw^{-1}$ d^{-1}.
These results indicate that:

- under the lower-bound approach, 0.1% of children exceed the toxicity reference value *(not statistically significant)* but no overexposure was observed in adults;
- under the upper-bound approach, 30% of adults and 74% of children exceed the toxicity reference value.

In the context of the TDS2, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) had concluded that a theoretical risk could not be ruled out for

these mycotoxins and had stressed the need to lower the analytical limits of quantification at least for the major contributors identified (pasta, and "bread and dried bread products").

On 30 November 2011, the European Food Safety Authority (EFSA) adopted an opinion on the risks for human and animal health related to the presence of T-2 and HT-2 toxins in food and feed. A tolerable daily intake (TDI) of 100 ng kg.bw^{-1} d^{-1} was established for the sum of these two toxins on the basis of the same pivotal study (Rafai, 1995) used for the PMTDI (JECFA, 2001) but using a 'Benchmark Dose' approach[1]. EFSA concluded that there was no health concern for these mycotoxins because the chronic exposure estimated for all categories of consumers is lower than the TDI of 100 ng kg.bw^{-1} d^{-1}.

On 7 February 2012, ANSES received a formal request from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) for an opinion on updating the results of the TDS2 relating to consumer exposure to T-2 and HT-2 toxins, in the light of the new TDI established by EFSA.

When investigating this matter, the Expert Committee on Assessment of the physical and chemical risks in food - *Evaluation des risques physiques et chimiques dans les aliments* (CES ERCA) - identified biases in the establishment of the new TDI.

This note explains the rationale behind the dissenting view of the CES ERCA concerning the TDI of 100 ng kg.bw^{-1} d^{-1} proposed by EFSA.

Organisation of the expert appraisal

ANSES entrusted the examination of this request to the Expert Committee on Physical and chemical residues and contaminants (CES RCCP) that has since become the Expert Committee on Assessment of the physical and chemical risks in food (CES ERCA).
This expert appraisal work was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities".

This expert appraisal work drew on an initial report written by an expert from the CES ERCA. It is based on data from EFSA (EFSA, 2011), JECFA (JECFA, 2001) and the SCF (SCF, 2001 and 2002) on the subject of T-2 and HT-2 mycotoxins, as well as on the toxicological studies by Rafai *et al.* (1995) and Meissonnier *et al.* (2008). It also provides information on the method used by EFSA to establish the Toxicological Reference Value (TRV) of 100 ng kg.bw^{-1} d^{-1}.

Result of the collective expert appraisal

The CES ERCA adopted the collective expert appraisal work that is the subject of this note, together with its conclusions and recommendations, at its meeting of 17 May 2013 and informed ANSES' General Directorate accordingly.

Review of the method used by EFSA to establish the TDI of 100 ng kg.bw^{-1} d^{-1}

After conducting a literature review of the new toxicological studies published since 2001, EFSA concluded that the study in piglets by Rafai *et al.* (1995) remains the most relevant

[1] The Benchmark Dose (BMD) is an alternative quantitative approach mainly for assessing dose-effect relationships from various animal experiments or epidemiological and observational studies. It corresponds to the dose leading to an excess risk level, set at 5 or 10%, of the selected critical effect. The Benchmark Dose Lower Confidence Limit (BMDL) is the lower limit of the 95% confidence interval for the BMD.

for establishing a TRV. The critical effect selected, namely the titre of anti-horse globulin antibodies on the 21st day of administration, remains unchanged compared to JECFA.

However, and in accordance with EFSA's Opinion of 29 June 2009, the benchmark dose approach was used to calculate a toxicological reference point for T-2 mycotoxins. Two families of models were used: exponential models and Hill models. The H2 model (Hill model with 2 parameters) was regarded as the most representative and enabled EFSA to derive a $BMDL_{05}$ of 10 µg.T-2 $kg.bw^{-1}$ d^{-1}.

After applying a safety factor of 100, a TDI of 100 ng $kg.bw^{-1}$ d^{-1} was then derived for the sum of T-2 and HT-2 mycotoxins.

The data used to calculate the $BMDL_{05}$ come from the publication by Rafai *et al.* (1995) and are summarised in the table below.

Dose µg $kg.bw^{-1}$ d^{-1}	Titre of anti-horse globulin antibodies (A-HG titre (log2))	Standard deviation	Number of piglets
0	6.30	0.84	10
0.029	4.50	0.84	10
0.062	5.15	0.90	10
0.105	4.30	1.17	10
0.129	4.10	0.70	10

Table 1: Experimental data from the publication by Rafai (1995) on the dietary exposure of piglets to T-2 and HT-2 mycotoxins

Remarks of the CES ERCA

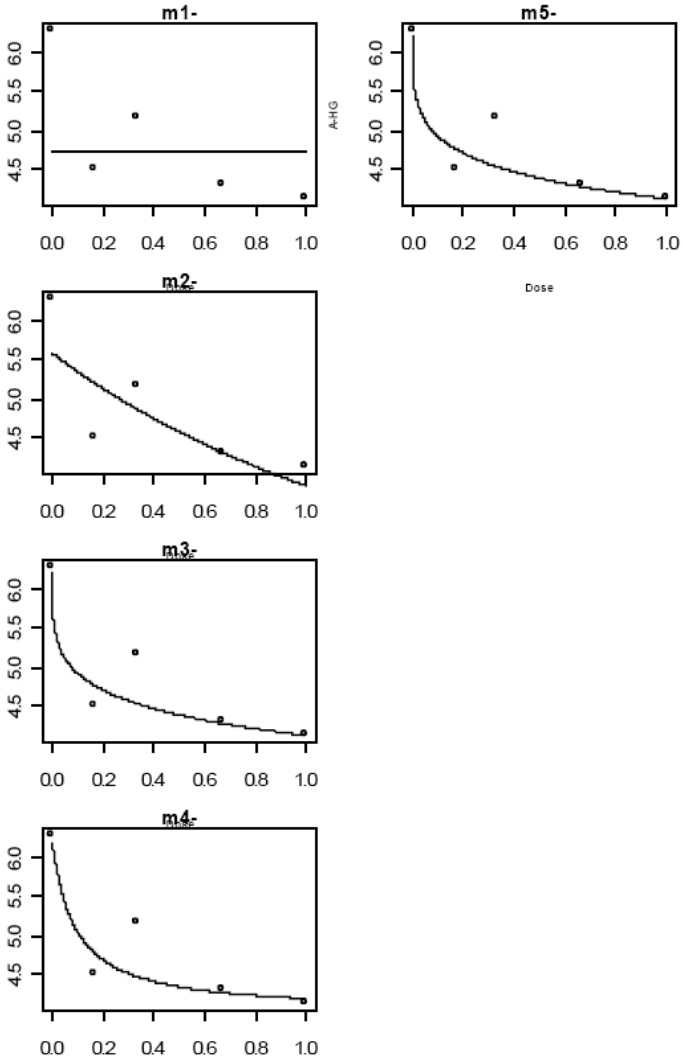
- **Concerning the use of the benchmark dose approach**

Since the mean titre value of the first dose is lower than that of the next dose (however, the difference is not statistically significant), it is difficult to fit the models applied by the benchmark dose approach.

After calculating BMDs using version 26.6 of PROAST software (used by EFSA for its modelling) and the Rafai data shown above, four Hill models were proposed (see table 2 below). From these, EFSA selected the M2 model. This curve was chosen by default because it is the only way to obtain a BMDL value. The other models lead to errors because their slope at the origin is too high, and the benchmark response (BMR) value does not intersect with the curve's confidence interval.

Hill models	Log (maximum likelihood)	BMD_{05} µg $kg.bw^{-1}$ d^{-1}	$BMDL_{05}$ µg $kg.bw^{-1}$ d^{-1}
Full (or reference)	14.23	-	-
M2	9.14	15	10.3
M3	11.05	Non-convergence	Non-convergence
M4	10.55	2.4	~0
M5	11.05	0.12	~0

Table 2: Results corresponding to the Hill models proposed by version 26.6 of the PROAST software to model the data from the publication by Rafai (1995)



Graph 1: Plots of curves corresponding to the Hill models proposed by version 26.6 of the PROAST software to model the data from the publication by Rafai (1995)

If the assumption is made that the benchmark dose approach is suited to the experimental data, it would have been more relevant to use models M4 and M5 because:

- the M2 model is not significantly representative of the experimental points (statistically different to the reference model) unlike the M4 and M5 models, which cannot however be used to obtain a $BMDL_{05}$;

- the M4 and M5 models fit the data better[2], as indicated by their likelihood criteria that are higher than those of the M2 model.

[2] The closer a model's logarithm of maximum likelihood is to that of the full model (called the reference model), the better the modelled curve fits the data.

The $BMDL_{05}$ selected by EFSA is based on a poor-fitting model that differs significantly from the reference model.
It should be noted that the purpose of choosing a BMDL is to enhance protection by lowering the BMD value into an area where the effect is very weak (less than 5%). However, according to the calculations, the BMD_{05} (15 µg $kg.bw^{-1}$ d^{-1}) and the $BMDL_{05}$ (10 µg $kg.bw^{-1}$ d^{-1}) obtained with the M2 model used by EFSA (see Table 2) are higher than the BMD_{05} values calculated using the M4 and M5 models (0.12 to 2.4 µg.$kg.bw^{-1}$.d^{-1}) which also fit the reference model better. There is currently no procedure available and generally accepted by users that addresses this issue (e.g. using the BMD determined from a model that fits the data better – even though this model does not provide an acceptable BMDL – rather than the BMDL from a model that does not fit the data as well but whose BMD is lower than the BMDL, or using an additional uncertainty factor, etc.).

- **Concerning the safety factors used to calculate the TDI**

According to EFSA, the uncertainty factors to be taken into account are inter-species variability (UF_A) and intra-species or inter-individual variability (UF_H) only, which leads to an overall factor of 100. In 2001, the SCF and JECFA had added a further factor of 5 for the use of a LOAEL.

In view of the difficulties encountered in calculating the BMDL, the uncertainty factor of 100 seems low, since it corresponds to an uncertainty applied when calculation of the BMDL is straightforward.

The CES ERCA considers that an additional safety factor would be necessary to compensate for the uncertainty related to calculation of the BMDL.

- **Concerning the use of averaged data (Rafai study)**

The experimental values obtained from the study by Rafai were the means for each group of 10 piglets receiving the same dose. This parameter should be considered as it affects the calculation of the BMD.

More recently, Meissonnier *et al.* also exposed piglets to T-2 and HT-2 mycotoxins in their feed (2008). There were five piglets per group (except for one dose where six piglets where exposed) compared with 10 in the Rafai study. The critical effect selected was once again the reduced production of anti-horse globulin antibodies on the 21st day of administration.

The data from the Meissonier study cannot be used to determine a TRV. However, in its opinion, EFSA proposed to compare the determination of the threshold based on the data from the Rafai study with the data from this study. Due to the lack of detail in the EFSA opinion the CES ERCA was unable to perform this comparison.

However, ANSES was able to acquire the individual data from the Meissonier study. Their analysis (described in the Annex 2) reveals that when individual data are used, this leads to the determination of a $BMDL_{05}$ that is 7 to 9 times lower than if it had been calculated from averaged data (as was the case with the results of the Rafai study). Hence, the $BMDL_{05}$ calculated from individual (but missing) data from the Rafai study would be lower than that determined by EFSA in its Opinion.

The CES ERCA considers that the lack of availability of individual data from the Rafai study contributes to the $BMDL_{05}$ being overestimated for T-2 and HT-2 mycotoxins.

Conclusions of the collective expert appraisal

EFSA used the benchmark dose approach to establish a tolerable daily intake of 100 ng $kg.bw^{-1}$ d^{-1} for all T-2 and HT-2 mycotoxins. Its modelling is based on averaged data from the experimental study by Rafai *et al.,* in which piglets were exposed to T-2 mycotoxins in their feed and their titres in anti-horse globulin antibodies were measured on the 21st day of administration.

The CES ERCA considers that the benchmark dose approach is problematic when using data from the Rafai study. Several biases have been identified, leading to the $BMDL_{05}$ being imprecisely estimated or even overestimated. If such an approach is used despite these caveats, an additional safety factor is essential to take account of the related uncertainty.

In conclusion, the CES ERCA believes that the dataset rules out the use of a benchmark dose approach. It considers that it would be more appropriate to base the risk assessment on the PMTDI of 60 ng $kg.bw^{-1}$ d^{-1}.

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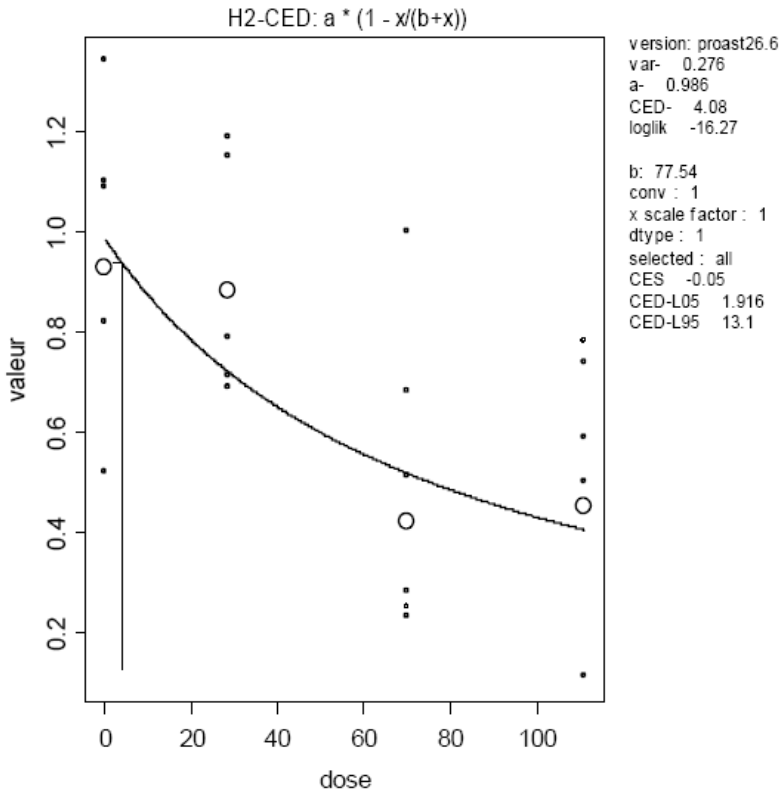
Date summary was validated by the Expert Committee: 17 May 2013

ANNEX 2
Observed differences in the calculation of BMD_{05} or $BMDL_{05}$ values depending on the type of data used (raw or averaged)

1- Calculation with raw data

In the study by Meissonier *et al.* (2008) there were five piglets per group (except for one dose where six piglets were used) compared with 10 in the study by Rafai *et al.* Moreover, information on exposure was not provided by Meissonnier *et al.* This can be calculated by analogy with the Rafai study by multiplying the dose in toxins expressed in µg kg^{-1} of feed by a coefficient of 53 to obtain exposure in µg $kg.bw^{-1}$. This value of 53 is a mean value based on Rafai's data and fluctuates between 43 and 62.

The resulting BMD_{05} and $BMDL_{05}$ values calculated with the raw data from the study by Meissonnier *et al.* are as follows:



The most appropriate model is a Hill model (known as H2: with 2 parameters). Its logarithm of maximum likelihood is -16.27 compared with -15.26 for the optimal full model. The BMD_{05} is 4.1 µg $kg.bw^{-1}$ d^{-1} and the $BMDL_{05}$ is 1.9 µg $kg.bw^{-1}$ d^{-1}.

The best exponential model (logarithm of maximum likelihood: -16.4) gives the following values: BMD_{05}: 6.5 µg $kg.bw^{-1}$ d^{-1} and $BMDL_{05}$ 4.0 µg $kg.bw^{-1}$ d^{-1}.

Logically therefore, the Hill model should be adopted.

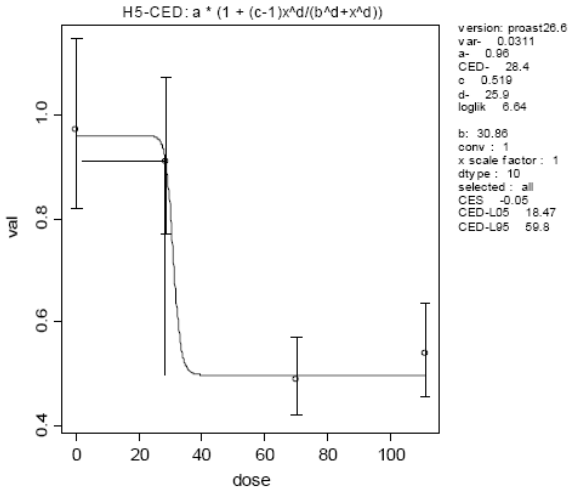
With the data from Rafai *et al.,* the $BMDL_{05}$ selected is five times higher (10 µg $kg.bw^{-1}$ d^{-1}) with certain methodological problems and values calculated from averaged data for each dose, whereas in this case the calculations were performed with raw data.

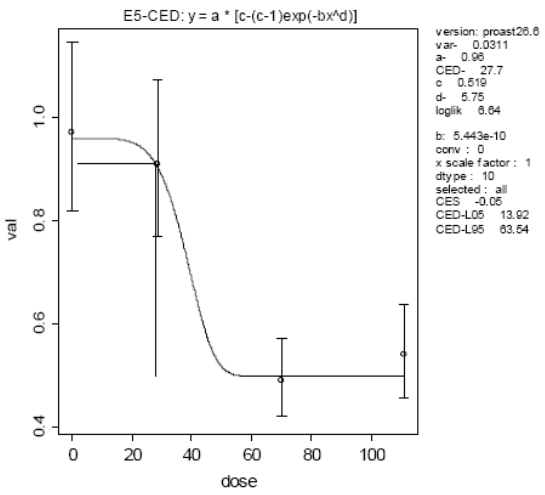
2- **Calculations with averaged data**

The model was recalculated, this time taking into account not the raw data but the averaged data from the Meissonier *et al.* study, to make it more comparable with the modelling performed using the data from Rafai *et al.,* and also to see the effect of using averaged data on the result when using a small number of samples.

The results are as follows:

Both the Hill model (H5) and the exponential model (E5) performed equally well, with a logarithm of maximum likelihood of 6.64 compared with an expected value of 7.11.





The BMD_{05} values are 28.4 and 27.7 µg $kg.bw^{-1}$ d^{-1} respectively, while the $BMDL_{05}$ values are 18.5 and 13.9 µg $kg.bw^{-1}$ d^{-1}, respectively.

The $BMDL_{05}$ is therefore 7 to 9 times higher than that obtained with the raw values (as shown above).

These data should certainly not be used, as they were obtained from a small number of datasets. When raw data are not used, the software assumes that the data follow a log-normal distribution (with mean values and standard deviations shown), which is a strong assumption when there are only five datasets per dose. This is also why the maximum likelihood (expressed as a log) differs so much between the (optimal) full models in the tests. In the first case, real data are used (log (maximum likelihood) = -15.26) while the other uses a model supposed to represent data based on an average and a standard deviation and a log-normal model (log (maximum likelihood) = 7.11).

Nevertheless, although this second model cannot be used, it may be useful for moderating the results obtained solely from the averaged data from Rafai et *al.* If there is a difference of 7 to 9 between the results obtained on raw data and the reduced data from Meissonier (with a sample size of 5), it is quite likely that large differences can be expected between the results obtained from raw data and the averaged data from the Rafai *et al.* study (perhaps slightly less because of the higher number of samples: 10).