FDA'S RESPONSE TO PEER REVIEW COMMENTS ON THE FDA'S TOXICOLOGICAL REFERENCE VALUE FOR CADMIUM (SEPTEMBER 2022)

November 2023

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I. INTRODUCTION

The U.S. Food and Drug Administration (FDA) considers several factors when evaluating whether dietary exposure from consumption of food with detectable levels of contaminants presents a potential health concern. Dietary exposure is estimated for the food containing the contaminant and then compared to the appropriate toxicological reference value (TRV). The TRV is typically derived from a point of departure (POD), that corresponds to a no or low observed adverse effect level (NOAEL) or LOAEL), to which appropriate uncertainty factors are applied. In the absence of an FDA derived TRV, peer-reviewed, publicly available TRVs from other regulatory agencies may be used. Currently, the existing TRVs for cadmium (Cd) derived by authoritative regulatory organizations differ considerably (ATSDR, 2012; EFSA, 2009; EPA, 1985; Leconte et al., 2021; WHO, 2011). In addition, when deriving these reference values, none of the regulatory organizations included internal validity [i.e., risk of bias (RoB)] evaluations for the supporting studies and the organizations used different mathematical models to derive their TRVs. Therefore, the FDA conducted an independent evaluation of the available data for Cd to derive an appropriate TRV for use in evaluating dietary exposure. We conducted a systematic review (SR) of the literature (Schaefer et al., 2022) to identify data related to critical adverse health effects associated with oral exposure to Cd and determined PODs based on these effects. We also reviewed, evaluated, and adapted human physiological based pharmacokinetic (PBPK) (Pouillot et al., 2022) models for use in reverse dosimetry analyses to relate internal measures of Cd exposure to external measures of exposure, specifically the PODs for Cd biomarkers to oral intake through food consumption. We made use of data identified from the SR and from the adapted models we developed as part of these efforts in deriving our TRV for Cd.

We used a weight of evidence approach, using multiple sources of information and lines of evidence, to develop a Cd TRV that is a range rather than a single value. We determined TRVs from both human and animal data with reverse dosimetry PBPK modeling and Benchmark Dose Modeling (BMD), respectively. We applied the PBPK model (with multiple models and for multiple health effects) for the human data [tubular degeneration, accumulation in the kidney cortex, decrease in bone mineral density (BoneMD)] to set the TRV range for Cd. The TRV derived from animal data using BMD modeling provided additional support for that range. This document provides a summary of the key studies and methods used by the FDA to derive oral TRV for Cd.

This peer review of the FDA's Toxicological Reference Value for Cadmium evaluates FDA's approach, including systematic review of the literature, models and assumptions, approach, and methods, as well as key findings and conclusions. For this peer review, three experts were independently selected by Versar Inc. to evaluate and provide written

comments on FDA's proposed Toxicological Reference Value for Cadmium (September 2022).

In Section II of this report, we list the charge questions given to the reviewers. In Section III of this report, we provide a summary of the general peer reviewer comments, and a description of major changes made to the report in response. In Section IV of this report, we provide a description of specific peer review comments and the changes made to the report in response. Finally, the individual peer reviewer comments are provided in tabular format in Appendix 1.

Below are the names and affiliation of the peer reviewers:

Gunnar F. Nordberg, M.D., Ph.D.
Umeå University
Department of Public Health and Clinical Medicine
Umeå, Sweden

Lars Barregard, M.D., Ph.D.
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II. CHARGE TO REVIEWERS

Charge Questions:

1. General Impressions - Provide overall impressions addressing the completeness of information presented and the clarity of presentation.

2. The derivation of the TRV focuses on oral cadmium exposure since this is the route of exposure to Cd from food. Have we adequately described the derivation method in sufficient detail for the work to be reproduced? If not, what additional information should we provide?

3. We conducted a comprehensive literature search of all publications on oral cadmium exposure up until 2020, and we relied upon and cited other sources that have extensively been reviewed in the literature. With this in mind, is there any additional pertinent literature that we should cite?

4. What are the strengths and limitations of the FDA approach to deriving the Cd TRV? Are there additional considerations for the FDA approach used?

5. We provide a range of values for the proposed TRV. Have we adequately explained the rationale for doing so? If not, what additional information should we provide?

6. We anticipate using the TRV to evaluate whether detectable levels of Cd in food is a potential health concern [i.e., an assessment where we compare the estimated exposure (level in the food x intake of the food) to the TRV]. Using this method, we would conclude that the estimated exposure to Cd at <0.3 µg/kg bw/day is not likely, 0.3 to <0.6 µg/kg bw/day may be likely, and >0.6 µg/kg bw/day is likely a health concern. Does the science support this characterization? If not, are there other considerations?

7. When deriving the TRV, we used a weight of evidence approach considering multiple lines of evidence including data from animals and humans. This information was used to determine a TRV range of 0.3-0.6 µg/kg bw/day, based on the PODs established from the epidemiologic studies (expressed in terms of urinary cadmium) acquired from the SR, the critical concentration of cadmium in the kidney cortex established from previous assessments, along with the reverse dosimetry model. The oral intake estimates were derived using reverse dosimetry from multiple comparable PBPK models assuming chronic exposure from birth to approximately 50 years of age. The PBPK models used were stochastic, including measures of parameter uncertainty and provided credible intervals for mean estimates. Additional uncertainty factors were not added. We also evaluated animal data, which provide additional information to consider when selecting the TRV. The draft report describes our methods for deriving the TRV. Have we sufficiently described our data assumptions, uncertainties, and limitations? If not, what specific additional information would improve the clarity.

8. OMB's memo M-19-15, Improving the Implementation of the Information Quality Act, notes that information disseminated by each federal agency be fit for its intended purpose, and that each agency considers the appropriate level of quality for each of the products it disseminates based on the likely use of that information.

The memo explains that quality encompasses utility, integrity, and objectivity. Given FDA's intended purpose of using the TRV to evaluate whether detectable levels of a Cd contaminant in food is a potential health concern, please comment on the utility, integrity, and objectivity of our draft report.

9. Do you have any other comments or suggestions?

III. SUMMARY OF PEER REVIEWER COMMENTS AND FDA RESPONSE

Question 1: General Impressions

Overall, the reviewers stated that the presentation of the work followed general scientific principles, is well described, clear and comprehensive. One reviewer thought the work was quite condensed because it required the reading of two companion papers. The reviewers thought the procedures used were in line with widely applied principles in risk assessment of chemical substances in food. One reviewer commented that this is the most elaborate work (compared to previous report by EFSA, JECFA and others) so far in translating the scientific knowledge from human and animal studies to a TRV (or health-based guidance value or tolerable intake).

FDA Response: Thank you for the thorough review of the FDA Cd TRV Draft and the companion papers. Your time and expertise are greatly appreciated.

Question 2: The derivation of the TRV focuses on oral cadmium exposure since this is the route of exposure to Cd from food. Have we adequately described the derivation method in sufficient detail for the work to be reproduced? If not, what additional information should we provide?

Overall, the reviewers thought the derivation method is well described. One reviewer noted that the description of the health effects of cadmium was short, but also recognized that it was covered by one of the companion papers (Schaefer et al., 2022).

FDA Response: Thank you for your comments.

Question 3: We conducted a comprehensive literature search of all publications on oral cadmium exposure up until 2020, and we relied upon and cited other sources that have extensively been reviewed in the literature. With this in mind, is there any additional pertinent literature that we should cite?

Overall, reviewers agreed that the literature search was comprehensive and adequate. One reviewer stated that in his opinion, the confidence today is higher for cardiovascular

effects (other than hypertension) than considered by the FDA in Schaefer et al. 2022, and that cardiovascular disease, at least coronary heart disease and stroke, deserved more attention. This reviewer also provided several additional cardiovascular resources to consider.

FDA Response: Thank you for your comments. As noted in the systematic review, for the cardiovascular endpoints in the animal data, we concluded that Cd exposure may affect cardiac tissue causing lesions, cell death, and inflammation. The initial confidence in the body of literature for the animal data on cardiovascular endpoints was considered moderate; however, more information is needed to establish a dose-response relationship. Thank you for the additional epidemiological references.

Question 4: What are the strengths and limitations of the FDA approach to deriving the Cd TRV? Are there additional considerations for the FDA approach used?

Reviewers listed the following strengths: A systematic review that considered the quality of epidemiological studies; an updated/mechanistic PBPK model for translating the U-Cd data to intake per kg/day; the report used both bone effects and kidney tubular effects in combination with animal data to support the range of TRV values; the report focused on deriving a range rather than a single value for oral cadmium exposure. A reviewer stated a range is much more informative in informing safety bounds than a fixed value because of variation in the response among individuals and populations.

Limitations provided by reviewers included: The TRVs use of LOAELS without any uncertainty factors; the use of the kidney cortex Cd concentration of 84 µg/g; too cautious assessment of the findings in the past decade on associations between Cd exposure and CVD.

One reviewer recognized that a NOAEL could not be derived from Akesson et al., 2005, because lowest category was the reference group, but thought this should result in the use of an uncertainty factor when using of a LOAEL, or alternatively, as done by ANSES, the upper limit of the reference group (or the preceding exposure category) could be used as a surrogate for a NOAEL.

The reviewer also stated that results from Engstrom et al., 2011, support the use of an uncertainty factor for LOAEL versus NOAEL because when osteoporosis (T-score below -2.5) was used as the outcome of interest, there was a significantly increased risk also in the middle tertile (0.59 µg/g creatinine) for hip and for hip+spine in all women as well as in never-smokers (Engstrom, Table 4).

Another reviewer questioned the value of the report being organized with human data presented first before animal data. This reviewer also desired more rationale for deriving the range for only female humans and suggested further clarification for this, or to derive a range for males.

FDA Response: Thank you for your comments on the strengths and limitations of FDA's approach. In considering the comments on uncertainties associated with the available data, we adjusted the choice of the POD as noted below.

Based on the reviewer's comment, we revised the choice of the BoneMD POD from the third tertile to the median U-Cd concentration in the second tertile (U-Cd, 0.50–0.75 µg/g median, 0.59 µg/g of creatinine), the mid-point of the middle exposure group for the POD. In Engstrom et al., 2011, the adjusted mean BoneMD was lower in the highest exposure category, with urinary cadmium ≥0.75 µg/g creatinine, median 0.87 µg/g creatinine as compared to the lowest category (<0.5 µg/g creatinine, median 0.30 µg/g creatinine). Thus, the U-Cd adjusted for creatinine (0.75 µg/g creatinine) in the third tertile was considered the "the lowest observed adverse effect level" (LOAEL) for a decrease in BoneMD. We agree that when osteoporosis (T-score below -2.5) is used as the outcome of interest instead of BoneMD, there is a significantly increased risk also in the middle tertile (0.59 µg/g creatinine) for the femoral neck in never-smoking women; however, the number of cases are limited. Therefore, we chose 0.59 µg/g of creatinine as the POD.

Based on the reviewer's comment, we revised the choice of the NAG POD (second quartile) and utilized the cadmium concentration (0.5 µg/L, 0.48 µg Cd/g creatinine) in the reference group as the NOAEL for our POD. Akesson et al., (2005) reported that the second quartile 0.50–0.75 µg/L U-Cd (0.79 µg/g creatinine) was significantly associated with effects on the renal tubules (as indicated by increased levels of NAG in urine), after adjusting for smoking, age, body mass index, blood lead, diabetes, hypertension, and regular use of nephrotoxic drugs when compared to the reference group [< 0.5 µg/L (0.48 µg cadmium/g creatinine)]. Therefore, the second quartile was considered the "LOAEL." Akesson et al., 2005, has no non-statistically significant exposure group relative to the reference group. Thus, we utilized the urinary cadmium concentration (0.5 µg/L, mean, 0.48 µg Cd/g creatinine) as a surrogate NOAEL for the POD to estimate the oral Cd intake that would lead to various levels of Cd found in the urine.

Taken together, we used the estimated POD of 0.50 µg/g creatinine based on the two observational studies (Engstrom et. al, 2011 and Akesson et al., 2005) for females aged 50-60 applied to the NHANES body weight model and creatinine model specified to the US population to determine the TRV range.

Therefore, in conclusion, we have revised the TRV range from 0.3-0.6 µg/kg bw/day to 0.21 µg/kg bw/day to 0.36 µg/kg bw/day. Using the Cadmium Reverse Dosimetry application, we determined that the range of 0.21 – 0.36 µg/kg bw/day would be protective of adverse kidney and bone effects based on 0.50 µg/g creatinine for females, the most sensitive population from Cd exposure (Table 1). Considering the cross-sectional nature of the study designs and since low-level U-Cd varies greatly within and between individuals, these variations may result in an overestimation of the risk from low-level Cd exposure (Åkesson et al. 2013) (RSI 2001). To further support the range, we used the upper bound estimate of the renal cortical concentration occurring in the U.S. population of 50 µg/g since the value is less than the projected critical concentration by a factor of four. We used reverse dosimetry based on the NHANES body weight model and creatinine model specified to the US population to determine the daily dose that would not exceed 50 µg/g in the renal cortex. The results indicate that if a population of women following a standard growth curve from NHANES is exposed to 0.36 µg/kg bw/day (confidence interval [0.28; 0.48]), every day from birth to 50 years of age, its mean renal cortex concentration will not exceed 50 µg/g cortex, (9447 µg in kidneys).

Updated Table 1 (TRVs Developed from epidemiological data) based on reviewer comments.

Endpoint	**Point of Departure**	**TRV[a]**
Bone (Engstrom, 2011)	U-Cd, 0.50–0.75 µg/g of creatinine (median = 0.59 µg/g creatinine)	0.23 (CI: 0.18-0.30) µg/kg bw/day for 64 yrs F
Kidney (Akesson, 2005)	U-Cd, upper limit of the reference group < 0.5 µg/L (mean = 0.48 µg/g creatinine)	0.22 (CI: 0.16-0.29) µg/kg bw/day for 58 yrs F
Combined Estimate from Bone and Kidney Endpoints (Engstrom, 2011 and Akesson, 2005)	Estimated POD = 0.50 µg/g creatinine	0.21 (CI: 0.16-0.29) µg/kg bw/day for **60** yrs F 0.28 (CI: 0.21-0.38) µg/kg bw/day for **50** yrs F
Kidney (EPA, 1985)	50 µg/g	0.36 (CI: 0.28-0.48) µg/kg bw/day
FDA Range	Estimated POD, U-Cd = 0.50 µg/g creatinine Concentration in Kidney Cortex = 50 µg/g	0.21 µg/kg bw/day to 0.36 µg/kg bw/day

[a] Kjellstrom Reverse dosimetry output from FDA-adapted Model #3 (and Nordberg (1978) model with Fransson et al. (2014)'s parameters (with C5 modified and C21 proportional to C19) and body weight model from NHANES) as described in (Pouillot et al., 2022)

With regard to the reviewer's suggestion to reorganize the order of presentation of the epi and animal studies. We first discussed the epidemiological data because those studies were used to develop the TRV range and then discussed the animal data because those studies are used to support the developed range.

With regard to the request for clarification about the use of female data. It would be more health-protective to base the range using data from the more sensitive sex, which is females. It is assumed there is a higher gastrointestinal absorption of Cd in women than in men (Akesson, 2019). The intestinal Cd absorption increases when there is a depletion in the body iron stores and at overt iron deficiency, which is a condition more prevalent in women at fertile age than in men (Genchi et al., 2020). As reported in Nordberg (2015), women with low body iron stores (serum ferritin values < 20 µg/L) the absorption was on average four times higher than that in subjects with normal stores (Flanagan et al., 1978).

Question 5: We provide a range of values for the proposed TRV. Have we adequately explained the rationale for doing so? If not, what additional information should we provide?

Overall, reviewers suggested minor clarifications of uncertainties and the use of the 'weight of evidence' approach. Additionally, one reviewer suggested FDA to reconsider the upper value in the range, because his opinion is that science does not support the "cut-off" value as high as 0.6 µg/kg bw/day (see Question 6).

FDA Response: Thank you for your comments. To clarify the Weight of Evidence Approach, additional detail was added to the manuscript under section 2.3 Derivation of the TRV.

As further explained in our response to Question 4, we have adjusted the upper range of 0.6 µg/kg bw/day to 0.36 µg/kg bw/day, based on 50 µg/g in the kidney cortex. This is because of the 22 studies included in Diamond et al., 2003 used to support the 84 µg/g K-Cd, 10 were included in the SR and 3 were included in our RoB analysis. All 3 were eventually excluded based on potential concern for bias in the 3 Key elements (confounding, exposure and/or outcome). Therefore, the cut-off value of 0.6 µg/kg bw/day (84 µg/g K-Cd) is not appropriate for use in the current model and analysis.

Question 6: We anticipate using the TRV to evaluate whether detectable levels of Cd in food is a potential health concern [i.e., an assessment where we compare the

estimated exposure (level in the food x intake of the food) to the TRV]. Using this method, we would conclude that the estimated exposure to Cd at <0.3 µg/kg bw/day is not likely, 0.3 to <0.6 µg/kg bw/day may be likely, and >0.6 µg/kg bw/day is likely a health concern. Does the science support this characterization? If not, are there other considerations?

One reviewer wanted FDA to reconsider the upper value in the range since the science does not support the "cut-off" value as high as 0.6 µg/kg bw/day. (see Question 5).

Another reviewer indicated that the range of 0.3-0.6 µg/kg bw/day is a safe range and that any adverse effects are highly unlikely. A third reviewer indicated that >0.6 µg/kg bw/day may be a health concern but should not be interpreted as indicating a high likelihood of cadmium related adverse health effects among persons without other risk factors.

FDA Response: We have removed the upper range of 0.6 µg/kg bw/day. Therefore, the upper range is now 0.36 µg/kg bw/day based on 50 µg/g in the kidney cortex. Because we have updated the range based on the comments, we have updated the characterization as follows: estimated exposure to Cd at <0.21 µg/kg bw/day is not likely, 0.21 to ≤ 0.36 µg/kg bw/day may be a health concern, and >0.36 µg/kg bw/day is likely a health concern. The TRV range will be used in our human health assessments as a benchmark to determine whether dietary exposure to Cd poses a potential health concern to consumers.

Question 7: When deriving the TRV, we used a weight of evidence approach considering multiple lines of evidence including data from animals and humans. This information was used to determine a TRV range of 0.3-0.6 µg/kg bw/day, based on the PODs established from the epidemiologic studies (expressed in terms of urinary cadmium) acquired from the SR, the critical concentration of cadmium in the kidney cortex established from previous assessments, along with the reverse dosimetry model. The oral intake estimates were derived using reverse dosimetry from multiple comparable PBPK models assuming chronic exposure from birth to approximately 50 years of age. The PBPK models used were stochastic, including measures of parameter uncertainty and provided credible intervals for mean estimates. Additional uncertainty factors were not added. We also evaluated animal data, which provide additional information to consider when selecting the TRV. The draft report describes our methods for deriving the TRV. Have we sufficiently described our data assumptions, uncertainties, and limitations? If not, what specific additional information would improve the clarity.

Each reviewer provided one or two suggestions. One reviewer reiterated clarifications on using the kidney cortex concentration of 84 µg/g as the POD for the upper bound of the TRV range and the rationale of not using any uncertainty factor for the PODs from the two human kidney and bone studies. Another reviewer stated that assumptions, uncertainties, and limitations are in most cases adequately described, but that urine flow should be mentioned in the report as a variation/uncertainty for U-Cd levels indicating Cd body burden, as should the relationship between B-Cd and urinary proteins from Akesson et al., 2005.

One reviewer also commented on the modeling, stating that the reverse dosimetry work in utilizing Model #3 of the paper due to Pouillot et al., whilst sound, is not stochastic. In addition, the importance of some model parameters should be checked using sensitivity analysis. Finally, the reviewer requested additional clarification of model functions.

FDA Response: Thank you for your comments. We addressed these comments by making specific changes to the report as described in Section IV. As stated earlier, we have addressed the concerns of the use of the kidney cortex concentration and the PODs by revising the TRV range to 0.21 µg/kg bw/day to 0.36 µg/kg bw/day.

In addition, while the model is not stochastic based on the strict definition the reviewer provides, the term is used to differentiate it from classical uses of the KN model that provide a single output for a given POD (backward) or exposure (Forward). As used in the Pouillot et al. paper, model #3 provides a distribution of outputs for a given POD or exposure. This distribution is linked to the uncertainty distribution from Fransson et al. parameters. By providing a distribution of values, it can be thought of as a stochastic model.

Question 8: OMB's memo M-19-15, Improving the Implementation of the Information Quality Act, notes that information disseminated by each federal agency be fit for its intended purpose, and that each agency considers the appropriate level of quality for each of the products it disseminates based on the likely use of that information. The memo explains that quality encompasses utility, integrity, and objectivity. Given FDA's intended purpose of using the TRV to evaluate whether detectable levels of a Cd contaminant in food is a potential health concern, please comment on the utility, integrity, and objectivity of our draft report.

All reviewers agreed the report is objective, displays integrity and is useful for its intended purposes. One reviewer thought that adverse health effects are likely to occur at somewhat lower levels than corresponding to long term U-Cd of 0.79 and 0.87 µg/g creatinine.

FDA Response: Thank you for your comments. Concerns that adverse health effects are likely to occur at somewhat lower levels were addressed in the previous comments.

Question 9: Do you have any other comments or suggestions?

One reviewer commented on the paper by Schaefer et al., 2022, with regards stability of biomarkers of exposure for Cd. Another reviewer had specific comments/corrections to be made in the report.

FDA Response: Thank you for your comments. We have addressed the specific comments/corrections in Section IV.

IV. SPECIFIC OBSERVATIONS ON THE FDA'S TOXICOLOGICAL REFERENCE VALUE FOR CADMIUM

Specific comments and concerns were addressed, and text changes were made to the report.

One reviewer stated that in his opinion, the confidence today is higher for cardiovascular effects (other than hypertension) than considered by the FDA in Schaefer et al. 2022, and that cardiovascular disease, at least coronary heart disease and stroke, deserved more attention in this report. Some of the additional epidemiological references suggested were considered but were not included because they did not meet the inclusion criteria and met exclusion criteria (i.e., a systematic review; exposure was not specific to dietary). FDA will continue to monitor the scientific data regarding dietary Cd exposure and adverse cardiovascular health effects. To further address this comment, we have added to the report under "Further Research Needed," "While the decrease in BoneMD, tubular degeneration, and accumulation of Cd in the kidney cortex were identified as the endpoints with the most robust data sets, other endpoints, such as cardiovascular effects from oral Cd exposure deserve further attention. As noted in Schaefer et a., 2022, we concluded that oral Cd exposure may affect cardiac tissue causing lesions, cell death, and inflammation in animals; however, more information is needed to establish a dose-response relationship. Fourteen epidemiological studies were also identified from the SR related to cardiovascular effects in humans after oral exposure to Cd. The studies primarily investigated relationships between Cd in blood and/or urine and the increased risk of stroke, myocardial infarction, carotid plaques, blood pressure, and heart failure. Overall, the evidence suggests that most studies with a positive association are related to levels of Cd found in the blood. Finally, Wu et al., 2022 recently developed a 3D cardiac organoid model that demonstrated that low-dose Cd exposure may inhibit cardiomyocyte

differentiation and cardiac induction. The FDA will continue to monitor the scientific literature regarding oral exposure to Cd and adverse cardiovascular effects."

One reviewer requested the use of Weight of Evidence Approach be clarified. To clarify the Weight of Evidence Approach, additional detail was added to the manuscript under section "Derivation of the TRV", "Using a weight of evidence approach, that is, considering all data described above, we estimated the Cd TRV..."

One reviewer requested clarification for text in Table 2, describing models in the text and adding "lowest Akaike information criterion" to Table 6. We added text to Table 2 for clarity. The models were added to the text for clarity and the Akaike Information Criteria was added to Table 6.

One reviewer thought that urine flow should be mentioned in the report as a variation/uncertainty for U-Cd levels indicating Cd body burden, given that there is no direct relationship between urine flow and glomerular filtration rate in early stages of renal tubular degeneration/dysfunction. We adjusted the sentence to include urine flow in the manuscript, "Finally, low-level U–Cd varies greatly within and between individuals, depending on several factors (*e.g.,* recent exposure, nutrition factors, glomerular filtration rate, and urine flow) (Bernard, 2016)."

In addition, this reviewer requested that we add that there were also statistically significant relationships between blood-cadmium and urinary N-acetyl-beta-glucosaminidase (NAG) and between blood-cadmium and urinary protein HC, strengthening the causality of the relationship between urinary Cd and urinary NAG. We added this information from Akesson et al. (2005) to the paper.

Another reviewer commented on the modeling, that the reverse dosimetry work utilizing Model #3 of the paper due to Pouillot et al., whilst sound, is not stochastic. We agree that the model is not stochastic based on the strict definition the reviewer provides, the term is used to differentiate it from classical uses of the KN model that provide a single output for a given POD (backward) or exposure (forward). As used in the Pouillot et al. paper, model #3 provides a distribution of outputs for a given POD or exposure. This distribution is linked to the uncertainty distribution from Fransson et al.'s parameters. By providing a distribution of values, it can be thought of as a stochastic model.

In addition, the reviewer questioned the importance of some model parameters (parameters (C7, C8, C16, C17, C19 and C20) identified in the Pouillot et al. paper) should be checked using sensitivity analysis. Fransson's et al. did a sensitivity analysis and, more importantly, an identifiability analysis that led to the choice of the parameters

they made some inferences on (C7, C8, C16, C17, C19 and C20). We adapted the model by using the Fransson et al. set of parameters.

The reviewer requested additional clarification of model functions (how *G(t)* and *A(t)* were defined mathematically in the ODEs (E1), (G1) and (Ex) of the Supplementary Information).

G(t) is time dependent dosing at each daily time point. The dose per kg of body weight is constant but because the weight is a function of time, G(t) is a function of time.

Finally, a reviewer noted editorial corrections to be made and to clarify that the Output/TRVs were informed by PBPK reverse dosimetry calculations. We performed the noted editorial corrections to the manuscript and added clarification to the table that that the Output/TRVs were informed by PBPK reverse dosimetry calculations.

V. APPENDIX

Individual Reviewer Responses to Charge Questions

CHARGE QUESTION 1: General Impressions - Provide overall impressions addressing the completeness of information presented and the clarity of presentation.

REVIEWER	COMMENT
Reviewer #1	My comments will cover not only the FDA TRV draft but also the two FDA papers (Schaeffer et al. 2002, Pouillot et al. 2022) that were used when deriving a TRV. I have focused on the human epidemiology data and the PBPK modelling in humans since my competence in the animal experimental methods is more limited. Overall, the presentation in the draft has a logical flow for readers familiar with this kind of risk assessment. It is very condensed (thus not so complete) and requires the reading of the two companion papers (Schaeffer et al. 2002, Pouillot et al. 2022), but I can understand that repeating too much of the content in the two published papers is not possible when the aim is to publish the report as a paper in a peer-reviewed scientific journal. Although I have some concerns as detailed below, this report is the most elaborate work (compared to previous report by EFSA, JECFA and others) so far in translating the scientific knowledge from human and animal studies to a TRV (or health-based guidance value or tolerable intake).
Reviewer #2	The “Draft FDA´s Toxicological Reference Value for Cadmium” (DFTRV) together with the supporting documents Pouillot et al 2022 and Schaefer et al 2022 represent a substantial work effort. It provides a complete coverage of the literature on bone effects and renal tubular effects up to 2020. It follows as far as the reviewer can evaluate the FDA principles for data handling as well as general scientific principles. The document is clear and easy to read. The systematic review (SR) and evaluation of the literature (Schaefer et al 2022) is based on a PECO (Populations, Exposure, Comparator/Control and Outcomes) statement, identifying decreased bone density and tubular degeneration in the kidney as critical effects. For other adverse

REVIEWER	COMMENT
	effects of cadmium (for example cancer, cardiovascular disease, reproductive effects, and endocrine disruption), Schaefer et al. refer to previous reviews by other Agencies and reviews by Akesson et al. 2014 and Nordberg et al. 2018. Based on the SR, Schaefer et al. identified studies with high quality and low risk of bias. The data handling seems thorough and adhering to the principles defined. The DFTRV authors used the high-quality studies as key studies when defining a cadmium POD (Point of Departure) for decreased bone density or tubular degeneration in kidneys respectively. PODs derived from the epidemiological studies were in terms of urinary cadmium levels. In order to obtain TRVs in terms of dietary intakes, the DFTRV authors used FDA adapted Physiologically Based Pharmaco-Kinetic models developed by Pouillot et al. 2022. Pouillot et al. 2022 amended previously published models by US-specific body weight, kidney weight and creatinine excretion from NHANES and WWEIA. Based on the amended models the DFTRV authors arrived at cadmium intakes corresponding to the POD values. The reviewer has not checked the calculations, but it all looks reasonable. The DFTRV authors then derived the TRV-range by a weight-of-evidence approach, using the epidemiological information supported by animal data. When integrating the animal data with the human/epidemiological data, the authors used uncertainty factors. The procedures used seem in line with previous FDA exercises in this field and are in line with widely applied principles in risk assessment of chemical substances in food. The derived TRV-range seems adequate.
Reviewer #3	The overall presentation of the work and manuscript is sound, and the work well described. The written word flows well, and explanations are clear. Please see my response to Question 9 below regarding minor corrections suggested. The rationale of bringing together a systematic review with PBPK modelling to derive the reported safe range for cadmium ingestion is good.

CHARGE QUESTION 2: The derivation of the TRV focuses on oral cadmium exposure since this is the route of exposure to Cd from food. Have we adequately described the derivation method in sufficient detail for the work to be reproduced? If not, what additional information should we provide?

REVIEWER	COMMENT
Reviewer #1	The derivation method has been described, though very shortly when it comes to the systematic review on health effects. But as noted above it is acceptable that this is covered by referring to Schaeffer et al. 2022. The PBPK method is described somewhat more in detail in the draft, and again, the issue is covered well by Pouillot et al. (2022).
Reviewer #2	The derivation method is well described.
Reviewer #3	The derivation is generally well described. I have the following observations/queries. - Table 2: It is not clear what is meant by "… from Equation D from the Ix et al. applied to …" which appears a number of times in the table. - At the bottom of page 17, mention is made of "all the available continuous models", but which models are these? Are they Models #1, #2 and #3 from Pouillot et al. or other ones? If not those from Pouillot et al., more detail is needed here. - At the bottom of page 17, mention is made of the "lowest Akaike information criterion", but I could not find this detail in Table 6.

CHARGE QUESTION 3: We conducted a comprehensive literature search of all publications on oral cadmium exposure up until 2020, and we relied upon and cited other sources that have extensively been reviewed in the literature. With this in mind, is there any additional pertinent literature that we should cite?

REVIEWER	COMMENT
Reviewer #1	The two outcomes used for derivation of the TRV in this draft are effects on the kidney (tubular damage) and bone (decreased bone mineral density). The choice of outcomes is based on a systematic review, which is described shortly, but in more detail in Schaeffer et al. 2022. The rationale for restricting the derivation of the TRV to kidney and bone outcomes is the "initial confidence in body of evidence", which is reasonable if this assessment is based on Nordberg et al. 2018, and previous assessment by ATSDR, EFSA and other agencies. In my opinion the confidence today is higher for cardiovascular effects (other than hypertension) than considered by the FDA in Schaeffer et al. 2022. After the assessments by ATSDR, EFSA, EPA and WHO, several systematic reviews have been published on incident cardiovascular disease, in particular coronary heart disease (CHD) and stroke (Tellez-Plaza et al. 2013, Chowdhury et al. 2018, Tinkov et al. 2018). All three reviews concluded that Cd exposure increases the risk of coronary heart disease and stroke. After the deadline of the FDA literature search further reviews have been published (Fagerberg and Barregard 2021). I also note that three prospective studies on CHD are missing in the list of cardiovascular outcomes in Table 6 of Schaeffer et al. 2022, namely Tellez-Plaza et al. 2012 (based on NHANES), Tellez-Plaza et al. 2013 (based on the Strong Heart Study), and Barregard et al. 2016 (based on the Swedish MDC study). Also, some cross-sectional studies are missing (e.g., Lee et al. 2011, Hecht et al. 2016), but these are less important since they likely have a higher risk of bias. In summary, cardiovascular disease, at least coronary heart disease and stroke, deserves more attention in the FDA draft. I realize that this comment relates mainly to the SR by Schaeffer et al. 2022, but since we are not asked to review that paper, I am commenting on the issue here.
Reviewer #2	The literature search and other sources seem adequate.
Reviewer #3	This is not an area I am familiar with in respect to my expertise in mathematical modelling, so I defer to others in this area. However, my opinion of the related Schaefer et al. review is that this appears comprehensive given the time period covered and number of references identified.

CHARGE QUESTION 4: What are the strengths and limitations of the FDA approach to deriving the Cd TRV? Are there additional considerations for the FDA approach used?

REVIEWER	COMMENT
Reviewer #1	The strengths are - a systematic review, considering also the quality of epidemiological studies - An updated PBPK model for translating the U-Cd data to intake per kg/day Limitations are - Use of LOAELS without any uncertainty factors (see a few lines below) - The less adequate use of the kidney cortex Cd concentration of 84 µg/g (see charge question #6) - A too cautious assessment of the findings in the past decade on associations between Cd exposure and CVD (see charge question #3) In the Introduction of the FDA draft the authors note that "The TRV is typically derived from a point of departure (POD), that corresponds to a no or low observed adverse effect level, to which appropriate uncertainty factors are applied". However, for the epidemiology data on effects on kidney and bone the FDA draft relies on two studies using Cd in urine, adjusted for creatinine (Akesson et al. 2005, Engstrom et al. 2011). In both cases the point of departure used was a LOAEL. It is understandable that a NOAEL could not be derived from these two studies since the lowest category was the reference group. But this should result in the use of an uncertainty factor taking into account the use of a LOAEL. Or, alternatively, as done by ANSES, the upper limit of the reference group (or the preceding exposure category) could be used as a surrogate for a NOAEL. This is especially relevant for the study by Engstrom et al. (2011). In the FDA draft and the paper by Schaeffer et al. (2022) the mean bone mineral density (BMD) was selected for the comparison between strata of U-Cd. But when osteoporosis (T-score below -2.5) was used as the outcome of interest, there was a significantly increased risk also in the middle tertile (0.59 µg/gC) for hip and for hip+spine in all women as well as in never-smokers (Engstrom Table 4). The number of cases was limited, but these results support the use of an uncertainty factor for LOAEL versus NOAEL when using the mean BMD as done by FDA.

REVIEWER	COMMENT
Reviewer #2	The FDA approach seems adequate. It is good to use epidemiological data for both bone effects and kidney tubular effects in combination with animal data to support the range of TRV values.
Reviewer #3	<u>Strengths</u> - The focus on deriving a range rather than a single value for oral cadmium exposure is good. Variation in the response between individuals in any population means, as this work has identified (as well as others), a range is much more informative in informing safety bounds than a fixed value. - The statistical work leading to identifying the main effects of cadmium toxicity being on bone and the kidneys is good and strengthens the application of mechanistic PBPK models. <u>Additional considerations</u> - I would normally expect consideration to be given to animal studies first followed by that of human ones, perhaps because I am used to seeing animal studies inform human ones. Is there any value to the manuscript being organized in this way, i.e., animal work reported before human work? - - It was unclear what the rationale was for deriving the range only for female humans. I was a bit surprised by this given the PBPK modelling has been formulated and parameterized for both sexes. Is there any danger here that the work will not be seen as significant when not reporting for both sexes? Perhaps this relates to where cadmium is applied in products/found in the environment? If so, this needs to be more clearly justified or the work needs to include the same assessment for males so it is inclusive of both sexes.

CHARGE QUESTION 5: We provide a range of values for the proposed TRV. Have we adequately explained the rationale for doing so? If not, what additional information should we provide?

REVIEWER	COMMENT
Reviewer #1	Yes, you have adequately explained the rationale. However, I am not convinced that the upper part of this range is valid, as explained below under Question #6.
Reviewer #2	The quantitative estimates are primarily from epidemiological observations, but at low urine-cadmium values uncertainties exist and it is good to give a range of TRV values indicating increasing likelihood of adverse effects. Some minor clarifications of uncertainties may be advisable (see #7)
Reviewer #3	Whilst the lead up to determining this range was clear, it was unclear what was meant by the phrase on page 22 “Using a weight of evidence approach, …”. How was this done? Was it achieved by looking at the lowest and highest safe bounds as identified by the systematic review and the modelling study or were these all combined in a particular way mathematically? Further detail here is needed.

CHARGE QUESTION 6: We anticipate using the TRV to evaluate whether detectable levels of Cd in food is a potential health concern [i.e., an assessment where we compare the estimated exposure (level in the food x intake of the food) to the TRV]. Using this method, we would conclude that the estimated exposure to Cd at <0.3 µg/kg bw/day is not likely, 0.3 to <0.6 µg/kg bw/day may be likely, and >0.6 µg/kg bw/day is likely a health concern. Does the science support this characterization? If not, are there other considerations?

REVIEWER	COMMENT
Reviewer #1	In my opinion science does not support the "cut-off" value as high as 0.6 µg/kg bw/day. This value is based on a kidney cortex Cd of 84 µg/g. The forward dosimetry, as described here and in Pouillot 2022 seems reasonable, but the kidney cortex concentration (K-Cd) of 84 µg/g is based on Diamond et al. 2003. In that paper Diamond et al. estimated which K-Cd (and dietary intake) resulted in a 10% risk of Cd-induced LMW proteinuria based on 22 studies. The estimate (mean) for K-Cd was 153 µg/g with a lower CI of 84 µg/g. For five general population studies the estimated mean was 31 µg/g. But the dose metric in almost all the 22 studies was U-Cd in µg/g. This was transformed to K-Cd using a PK model, and this model was the one named Model #1 in Pouillot et al. 2022. Since the FDA draft and Pouillot et al. 2022 has found Model #3 to be superior with a more valid estimate of the ratio K-Cd/U-Cd it is not logic to use the 84 µg/g value from Diamond et al. 2003. Using the 60:1 ratio from Akerstrom et al., this kidney cortex concentration corresponds to 1.3 µg/gC, which is higher that the POD selected by FDA from the study by Akesson et al. 2005.
Reviewer #2	>0.6 µg/kg bw/day may be a health concern but should not be interpreted as indicating a high likelihood of cadmium related adverse health effects among persons without other risk factors.
Reviewer #3	I think characterizations like this are hard to make and I am conscious of the effect of the wording. For instance, it would appear to me that the range of 0.3-0.6 µg/kg bw/day, given all the evidence provided in the manuscript, is a safe range and that any adverse effects are highly unlikely. Indeed, I like the phrasing in the manuscript of "Therefore, based on the current available evidence, there is high confidence that the range of the proposed TRV of 0.3-0.6 µg/kg bw/day will be protective to human health." What is hard to characterize here is what "may be likely" actually means. The science supports this idea, but I am concerned wording like "and >0.6 µg/kg bw/day is likely a health concern" may be interpreted as it is ok to go above this value, the issue being it is not clear the increased risk of doing so for a given dose about this value for a given time period.

CHARGE QUESTION 7: When deriving the TRV, we used a weight of evidence approach considering multiple lines of evidence including data from animals and humans. This information was used to determine a TRV range of 0.3-0.6 µg/kg bw/day, based on the PODs established from the epidemiologic studies (expressed in terms of urinary cadmium) acquired from the SR, the critical concentration of cadmium in the kidney cortex established from previous assessments, along with the reverse dosimetry model. The oral intake estimates were derived using reverse dosimetry from multiple comparable PBPK models assuming chronic exposure from birth to approximately 50 years of age. The PBPK models used were stochastic, including measures of parameter uncertainty and provided credible intervals for mean estimates. Additional uncertainty factors were not added. We also evaluated animal data, which provide additional information to consider when selecting the TRV. The draft report describes our methods for deriving the TRV. Have we sufficiently described our data assumptions, uncertainties, and limitations? If not, what specific additional information would improve the clarity.

REVIEWER	COMMENT
Reviewer #1	The approach has been well described, as have the assumptions. The PBPK models used are well described, and the considerations here and in Pouillot et al. 2002 regarding body weight, creatinine excretion, and the "best" modifications of the original KN model are carefully elaborated. My concern about the TRV of 0.6 µg/kg bw/day and my opinion on the lack of an uncertainty factor have been described under questions #6 and #4. So, further clarifications would be welcome on a) the possible inconsistency regarding the kidney cortex concentration of 84 µg/g and the use of Model #3 in the PBPK modelling and the 60:1 ratio and b) the rationale of not using any uncertainty factor for the two human kidney and bone studies.
Reviewer #2	Assumptions, uncertainties, and limitations are in most cases adequately described. Some small clarifications/additions may be useful: Page 7 in the DFTRV document, lines 9-11 describes the variation/uncertainties in urinary cadmium levels as an indicator of cadmium body burden, referring to Bernard et al. 2016, who pointed out the importance of urine flow (diuresis). These uncertainties are also described in section 6.1.2 of the Cadmium chapter in the Handbook on the Toxicology of Metals, 5th Ed, 2022 (appended). Urine flow is not mentioned in the text on line 7 in page 7 of the DFTRV and should be added. Please note that there is no direct relationship between urine flow and glomerular filtration rate in early stages of renal tubular degeneration/dysfunction.

REVIEWER	COMMENT
	Page 9 describes the epidemiological data on renal tubular degeneration in relation to urinary cadmium and the DFTRV authors derived 0.79 µg/g creatinine as a POD. Because of the uncertainties of the relationship between urinary cadmium and cadmium body burden, pointed out by Bernard et al. 2016 and others, it would be of value to add some information strengthening the causality of the relationship. On page 9 line 16, please add the following information from Akesson et al. 2005: There were also statistically significant relationships between blood-cadmium and urinary NAG and between blood-cadmium and urinary protein HC, strengthening the causality of the relationship between urinary Cd and urinary NAG.
Reviewer #3	I have the following points in respect of the modelling. - The reverse dosimetry work utilizing Model #3 of the paper due to Pouillot et al., whilst sound, is not stochastic. The realization of the system of inhomogeneous linear ordinary differential equations (ODEs) for certain parameter value distributions, means the system being solved is still deterministic. It may well be that a range of dosing outcomes are then derived (and one would normally expect this), but to be stochastic either the rates per time step would need to change in time or a Wiener type process would need to be added to the governing ODEs. - Whilst local sensitivity analysis of Model #1 and Model #2 was undertaken in the paper due to Fransson et al., it is not clear to me how the importance of the parameters (C7, C8, C16, C17, C19 and C20) identified in the Pouillot et al. paper, which informs this work, have been determined given Model #3 changes the structure of the underlying model. As such, a sensitivity analysis should be conducted here to identify the importance of the respective parameters. It may well be the same parameters are identified, but this should be checked. This will then satisfy any reasons as to why the said list of parameters were chosen, specifically in terms of considering parameter distributions and improve confidence in the model predictions. It will also help to highlight any other parameters which may be important in affecting the dosing outcome.

REVIEWER	COMMENT
	- From the paper due to Pouillot et al., it was not clear to me how *G(t)* and *A(t)* were defined mathematically in the ODEs (E1), (G1) and (Ex) of the Supplementary Information of that paper. For instance, dosing is discussed as being daily, but simulations are over a very long time period, i.e., many decades. As such, is the gut dosing undertaken by time dependent instantaneous dosing at each daily time point or given the long period of time considered, is this dosing not just assumed to be constant?

CHARGE QUESTION 8: OMB's memo M-19-15, Improving the Implementation of the Information Quality Act, notes that information disseminated by each federal agency be fit for its intended purpose, and that each agency considers the appropriate level of quality for each of the products it disseminates based on the likely use of that information. The memo explains that quality encompasses utility, integrity, and objectivity. Given FDA's intended purpose of using the TRV to evaluate whether detectable levels of a Cd contaminant in food is a potential health concern, please comment on the utility, integrity, and objectivity of our draft report.

REVIEWER	COMMENT
Reviewer #1	The utility and integrity are satisfactory. The draft report is also an objective product, but as outlined in the comments to charge questions 4 and 6, I think the approach is too conservative – that is adverse health comments to effects are likely to occur at somewhat lower levels than corresponding to long term U-Cd of 0.79 and 0.87 µg/g C.
Reviewer #2	The draft report seems, as far as the reviewer can evaluate, useful for the intended purposes, it uses FDA principles of risk assessment for food, thus establishing its integrity. Documentation of its objectivity rests on the systematic review of the original scientific literature and previously published scientific reviews and the use of established risk assessment principles, previously developed by FDA.
Reviewer #3	I believe the utility, integrity and objectivity of this draft report is sound and in line with the way in which the derivation of such information should be reported.

CHARGE QUESTION 9: Do you have any other comments or suggestions?

REVIEWER	COMMENT
Reviewer #1	As a minor comment to the text on B-Cd in the paper by Schaefer et al. (2022), I would like to state that, yes, B-Cd mirrors recent exposure better than U-Cd. But if exposure is relatively stable, then B-Cd also mirrors the body burden of cadmium (without the problems of other factors than Cd body burden affecting U-Cd). The main problem with B-Cd as a long-term exposure marker is the first year after people have started or stopped smoking. This is also expressed in the review by Nordberg et al. (2018): "After long-term cadmium exposure, an increasing proportion of blood cadmium will be related to the body burden, and blood cadmium is a good indicator of internal dose and accumulation in the kidney and other soft tissues in long-term orally exposed population groups". This is well illustrated by Hecht et al. (Biomarkers 2016) in former smokers. There was even a higher correlation between B-Cd and duration of smoking or pack-years than the correlation between U-Cd and these smoking metrics.
Reviewer #2	No other comments.
Reviewer #3	In Table 10 it would be helpful to make it clear that the Output/TRVs were informed by PBPK reverse dosimetry calculations, so that the distinction between the human studies and PBPK modelling results are clear and can be easily compared in the one place in the manuscript. I have noted the following corrections. - Page 17: "… data in Tables 4 and 5, where were …" - Page 21: "Mitsumori et al. (1998) and Shibutani et al. (2000):" - Page 22, Table 9: I would suggest "ERROR – no viable models" is replaced with "No viable models". An explanation as to why this is the case should also be provided. - References: Some of the references use full journal names, whilst others use abbreviations.