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Posted Date: 9 February 2026

doi: 10.20944/preprints202602.0599.v1

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Article

Predicting Human Aluminium Exposure from Vaccinations Using a Physiologically-Based Toxicokinetic Model

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Abstract

Background/Objectives: Poorly soluble aluminium (Al) compounds have successfully been used for decades as adjuvants in vaccines enabling an effective immune response. Yet the safety of Al exposure from vaccines is consistently questioned, especially regarding infants. Since toxicokinetic data of aluminium after vaccination in humans are not available, model-informed predictions are needed for risk assessment.

Methods: Using a physiologically-based toxicokinetic model we predicted the Al exposure from i.m. injections of Al-adsorbed vaccines for full-term neonates to 50-year-old adults following the recommended vaccination schedule in Germany 2025 in addition to the continuous oral background Al exposure from dietary intake.

Results: During the first two years of life, moderate (max. 2 to 3-fold) but transient increases of Al concentrations in plasma and in the relevant target organs liver and bone due to vaccinations were predicted. Increase in brain Al content (4%) was minimal. Most importantly, in all tissues, maximum Al levels did not exceed normal levels observed in infants soon after birth or known from adults. In children and adults, the rise in Al concentrations in plasma and tissues due to single vaccinations was marginal. The calculated contribution of vaccinations to the Al body burden at age 50 was negligible. **Conclusions:** From a toxicokinetic perspective, the additional Al exposure in full-term infants, children and adults from vaccinations with Al-adsorbed vaccines according to the current recommended schedules is considered safe. The model has proven a valuable tool for predictions of Al exposure from vaccinations.

Keywords: aluminium; adjuvants; immunological; toxicokinetics; vaccination; computer simulation

1. Introduction

Many important vaccines are adsorbed on aluminium (Al) adjuvants, e.g. the toxoid vaccines against diphtheria and tetanus, acellular pertussis, hepatitis B, pneumococcal and meningococcal vaccines. Al adjuvants potentiate the immune response to the poorly immunogenic antigens and thereby enable a successful vaccination. In Europe, the content of Al in vaccines is limited by the European Pharmacopoeia to a maximum of 1.25 mg Al per dose [1].

Al compounds used as vaccine adjuvants mainly consist of complex polymers of crystalline aluminium oxyhydroxide or amorphous aluminium hydroxyphosphate [2], referred to below for the ease of reading as AH (Al hydroxide), AP (Al phosphate), and AAHS (amorphous Al

hydroxyphosphate sulfate). Vaccine antigens are typically adsorbed onto the surface of preformed adjuvants [3]. After vaccination the amount of injected Al can be considered as 100% systemically available, but due to poor solubility intramuscular absorption is very slow. Both amorphous structure and higher solubility might contribute to the higher rate of systemic availability of AP compared to AH. Estimates from injection site measurements suggest that complete absorption takes about 3 months (AP) up to 1 year (AH) [4].

Al-containing vaccines have been used successfully and safely over decades [5,6]. The existing evidence has been supplemented recently by an epidemiological study from Denmark [7] that did not find evidence supporting an increased risk for autoimmune, atopic or allergic, or neurodevelopmental disorders associated with early childhood exposure to aluminium-adsorbed vaccines. Nevertheless, remaining concerns have been raised considering the fact that neonates and infants receive the same (or even higher) absolute Al amounts per vaccination than adults during the first year of life, while their kidney function, which is essential for elimination of Al from the body, is immature.

The concerns are compounded by the fact that the development of new vaccines and adapted recommendations over 40 years lead to a steady increase of Al exposure from vaccinations during the first two years of life. For example, the cumulative Al exposure via vaccination has increased 3 to 4 times in Germany since 1980 depending on the chosen brand.

Attempts to assess the risk based on comparison of cumulative amounts of Al exposure from various sources (e.g. by [8]) are limited because they do not account for i) the rates of absorption from various routes of exposure (apart from the extent of absorption as 0.1-0.3% from diet vs. 100% from vaccines); ii) the kinetic differences between a continuous intake of low amounts (as exposure from diet) and a singular administration of a high dose (as with vaccination), even if cumulative amounts are equal; iii) the age-dependent physiology influencing Al kinetics; and iv) the variable impact of an increase in (e.g. cumulative) exposure depending on the Al load reached in relevant target organs.

Physiologically-based toxicokinetic modelling allows to overcome these difficulties by extrapolating from diverse kinetic data to specific predictions of exposures, esp. in children and infants. Moreover, a model-informed assessment can continuously be adapted, e.g., when Al content and vaccination schedules are subject to change.

In recent years we have closed the scientific gap identified in 2007, i.e. the absence of a physiologically-based toxicokinetic (PBTK) model being capable of predicting both plasma and tissue concentrations with sufficient accuracy [9]. We have developed a PBTK model for Aluminium exposure in humans that is built on a comprehensive collection of animal and human toxicokinetic data [4,10]. The model could successfully predict data from animals and humans after s.c and i.m. absorption from adjuvanted vaccines or immunotherapeutics [4]. It was recently applied to simulate Al exposure from subcutaneous immunotherapy (SCIT) in different age groups [11].

Herein we leverage the PBTK model to predict the joint Al exposure from diet and vaccinations from birth onwards comparing Al concentration time courses as well as the accumulated Al body burden over lifetime. The predictions are intended to provide support for regulatory risk assessment and a science-based contribution to the discussions on the safety of Al in vaccines.

2. Materials and Methods

2.1. Simulation Scenarios

2.1.1. Al Exposure from Dietary Intake

As described and used recently [4,11], a continuous age-dependent dietary intake scenario was simulated to generate background exposure levels of Al expected in healthy female subjects from birth to adulthood (50y). In brief, based on results of European diet studies [12], an oral exposure of 0.8 mg Al/kg/week from food and water was assumed for children and adults (> 12 months), and of 0.1, 0.2, 0.4 and 0.8 mg/kg/week for infants aged 0-3, 4-6, 7-9 and 10-12 months, resulting from infant

formulae and other foods manufactured especially for infants. Further assumptions include an average oral bioavailability of 0.17% [4], and initial Al levels at birth built up in all organs during embryonic development (see 2.2).

This dietary intake scenario was used as the basis for simulations of additional Al exposure from vaccinations. As Al in food is the main source of dietary exposure the term “food only” is used in the following.

2.1.2. Al Exposure from Food and Vaccinations from Birth to Adulthood (0-50y)

Al exposure from vaccinations was simulated according to the vaccination schedule recommended for the whole population between 0 and 50 years of age in Germany 2025 ([13]; Table 1, white area). Vaccinations for special risk groups (known as indicated vaccinations) or for traveling purposes were not considered. Only vaccinations that contain Al adjuvants were included, thus not included are recommended vaccinations with live attenuated vaccines (e.g. measles containing vaccines) or others not containing Al (e.g. inactivated influenza vaccines). For vaccination against human papillomavirus (HPV), the recommended 2-dose scheme was applied. All vaccinations were administered at the earliest age in the recommended period. In the case of more than one available product for a particular vaccination on the market in Germany 2025, the one with the higher Al content was used. Adjuvant type (AH: Aluminium hydroxide; AP: Aluminium phosphate; AAHS: Amorphous Aluminium hydroxyphosphate sulfate) and Al amount per vaccine dose of the products were retrieved from Section 2 of the summary of product characteristics (SmPC). Al exposure from vaccinations was predicted in addition to the background exposure from food (“food+vacc”; see 2.1.1).

Table 1. Al exposure from vaccinations from birth to 50 years of age used in the simulation scenarios based on the vaccination schedule 2025 [13] and the highest Al amount of available products in Germany; Al adjuvant type (AH: Aluminium hydroxide; AP: Aluminium phosphate; AAHS: Amorphous Aluminium hydroxyphosphate sulfate) and Al amount per vaccine dose were retrieved from the Summary of Product Informations.

Age		Vaccination	Al amount (mg) per vaccine dose			Total Al dose
month	year		AH	AP	AAHS	
0	0	HepB			0.25	0.25
1	0.08	HepB			0.25	0.25
2	0.2	DTaP-IPV- Hib-HepB	0.5	0.32		0.82
2	0.2	PCV13/15		0.125		0.125
2	0.2	MenB	0.5			0.5
4	0.3	DTaP-IPV- Hib-HepB	0.5	0.32		0.82
4	0.3	PCV13/15		0.125		0.125
4	0.3	MenB	0.5			0.5
11	0.9	DTaP-IPV- Hib-HepB	0.5	0.32		0.82
11	0.9	PCV13/15		0.125		0.125
12	1.0	MenB	0.5			0.5
12	1.0	MenC	0.5			0.5
60	5	TdaP	0.3	0.2		0.5
108	9	HPV (1 st)			0.5	0.5
108	9	TdaP-IPV	0.3	0.2		0.5
120	10	HPV (2 nd)			0.5	0.5
228	19	TdaP	0.3	0.2		0.5
348	29	Td	0.5			0.5

468	39	Td	0.5			0.5
588	49	Td	0.5			0.5
		Total	5.9	1.9	1.0 (1.5)	8.8 (9.3)

2.1.3. Al Exposure from Food and Vaccinations Including “Additional HepB at Birth”

Since vaccines against hepatitis B (HepB) are also Al-adjuvanted, and in some EEA (European Economic Area) countries [14] there is a general recommendation for HepB vaccination at birth, we added a second scenario with two additional HepB vaccinations at birth and 1mo (see Table 1, grey area). In Germany, as in most European countries, this schedule is only recommended for newborns of HepB infected mothers. Two monovalent HepB vaccines for children using either AH or AAHS are available. For the predictions, we assumed an AAHS vaccine due to the faster absorption rate as the most conservative approach.

2.1.4. Body Burden of Al from Food and Vaccinations After 50 Years of Life

In order to investigate the contribution of Al from vaccinations to the Al body burden at later ages we calculated the total amount of Al present in the body of a reference female adult at the age of 50y for both scenarios (food only and food+vacc including “additional HepB at birth” from 0-50y).

2.2. PBTK Model Structure and Simulation Details

Simulations were based on the PBTK model described in Hartung et al. 2025 [4]. This model is characterized by an age-dependent physiological parametrization for full-term newborns to adults, combined with a model for GFR (glomerular filtration rate) maturation, and a unique bone submodel. In this submodel, Al uptake into bone is assumed to be proportional to calcium (Ca) uptake, and Al release from bone identical to that of Ca [15]. The Ca kinetic submodel captured these age-dependent bone remodelling processes from birth to adulthood. Brain is conservatively modelled as a sink due to the available experimental data base that shows no sign of release from the tissue [10].

In contrast to the recent simulations for SCIT [11], a particular focus here is on the suitability of the model for predictions in newborns and infants shortly after birth. Apart from the inclusion of two age-dependent physiological processes most relevant for Al kinetics, maturation of renal function and bone remodelling (see above), the model also accounts for the impact of the fetal Al exposure during pregnancyby assuming initial Al levels in all organs at birth . Organ-specific initial level distributions have previously been fitted to literature data from multiple sources [4]. In order to be fully in line with the plasma level predicted in our female adult after food only exposure ensuring quasi steady state conditions at birth we had to slightly reduce the median initial level in plasma from 5.5 to 2.2 µg/L. This is at the lower end of the range of plasma levels of newborns reported in literature (2-15 µg/L; [4]). Compared to higher initial levels this is considered a more conservative approach, since relative increases due to vaccination are more pronounced.

Inter-individual variability is implemented on model parameters (oral bioavailability, GFR and tissue distribution; [4]). The model further contains dosing modules for oral and parenteral routes of administration. For i.m. absorption of Al from adjuvants, a specific zero-order absorption rate was used for each adjuvant type (AH: 0.002784/day, AP: 0.01098/day) estimated from injection site release data after adjuvant administration in various animal species (rat, rabbit, monkey; see [4]). Since AAHS can be considered a form of AP adjuvant with similar physicochemical and dissolution properties [3], the same rate was applied as for AP. The i.m. absorption rates were assumed to be lognormally distributed in the population, with a 50% coefficient of variation [4].

Simulation results and statistical analyses were obtained using the software R, version 4.2.2 [16], specifically using the R package mlxR version 4.2.0 [17] for Al exposure predictions in tissues and plasma.

For each simulation, we used an identical virtual population of N=500 female individuals to ensure comparability amongst the scenarios. Predictions for both sexes confirmed that female subjects can be considered the toxicologically more sensitive population (see Figure S1 in [11]).

2.3. Data Retrieval and Evaluation

The predicted Al concentration-time curves after food+vacc exposure are displayed as median with lower (5% quantile, p5) and upper (95% quantile, p95) bounds. We graphically superimposed the food+vacc exposure levels on the median exposure expected from food only (dotted line) to highlight the increase in Al exposure attributed to vaccinations. Al concentrations (median, p95) in plasma and tissues were calculated at the timepoint of the highest median difference during infancy (0-2y), between 9-10y (after first HPV and Tdap-IPV booster vaccination), and 19-20y (after Tdap booster vaccination). For all concentrations the differences and ratios of the corresponding medians after food+vacc compared to food only were calculated.

The toxicological evaluation of predicted total Al levels in tissues was based on data of upper limits of normal (ULN) or critical levels of Al in tissues reported in literature. As done in [4,11], values published as $\mu\text{g/g}$ dry weight (dw) were converted into Al amounts per wet weight (ww) to allow for a meaningful comparison with predicted Al tissue levels modelled as ww. For example, the conversion factor for bone samples (0.497) accounts for the difference between Al in dry bone samples and predicted Al concentrations in wet model bone tissue defined as cartilage-free and bone marrow-including (for details see [4] (chapter 2.2.3)).

A level of 5 $\mu\text{g/g}$ ww was considered as ULN for Al content in bone of healthy adults (converted from the ULN of 10 $\mu\text{g/g}$ dw reviewed by [18]). Levels > 30 $\mu\text{g/g}$ ww are reported to be clearly associated with Al-induced bone disease and osteomalacia [18–20]. The lowest Al content in bone associated with osteomalacia symptoms (7 $\mu\text{g/g}$ ww, herein referred to as “critical level”; converted from 14 $\mu\text{g/g}$ dw) was found in one adult patient on long-term total parenteral nutrition (TPN) which constitutes a relevant source of Al due to contaminated PN solutions [21].

A derivation of a ULN is more uncertain for brain tissue. A range of 0.02-1 $\mu\text{g/g}$ ww for ‘normal’ brains over several decades is reported (converted from 0.1-4.5 $\mu\text{g/g}$ dw [22]); conversion factor 0.23, see [4]). Given the available data we herein refer to 1 $\mu\text{g/g}$ ww as a ULN for Al in brain of adults. A critical level of Al content in brain with respect to neurotoxicity is unknown.

A ULN of 4 $\mu\text{g/g}$ ww in liver for adults was deduced from the range of 0.07-4.33 $\mu\text{g/g}$ ww measured in 140 adult autopsy liver tissues in Germany (age range 21-81y, [23]). Measurements in children showing hepatotoxic symptoms after TPN (18 to 34 months of age) were in the range of 8-40.5 $\mu\text{g Al/g}$ ww (converted from 32-162 $\mu\text{g/g}$ dw, [24]; conversion factor 0.25, see [4]), but the hepatic pathology observed was not clearly related to Al exposure from TPN only. Therefore, critical levels for the Al content in liver also cannot be inferred.

All ULN as well as toxicity-associated levels derived are displayed in the figures as horizontal dotted or solid line, respectively.

Available data on tissue Al levels in healthy newborns and infants had been used to estimate initial levels at birth ([4]; see 2.2.).

3. Results

3.1. Al Exposure from Food and Vaccinations from Birth to Adulthood (0-50y)

Figure 1 shows the predicted time courses of Al concentration in plasma and tissues from birth to 50 years of age following joint exposure from vaccinations recommended for the general population in Germany 2025 and dietary intake (food+vacc) compared to the median of the background dietary intake only (food only). The quantiles of the predicted time courses after food only exposure for all tissues are shown elsewhere [11].

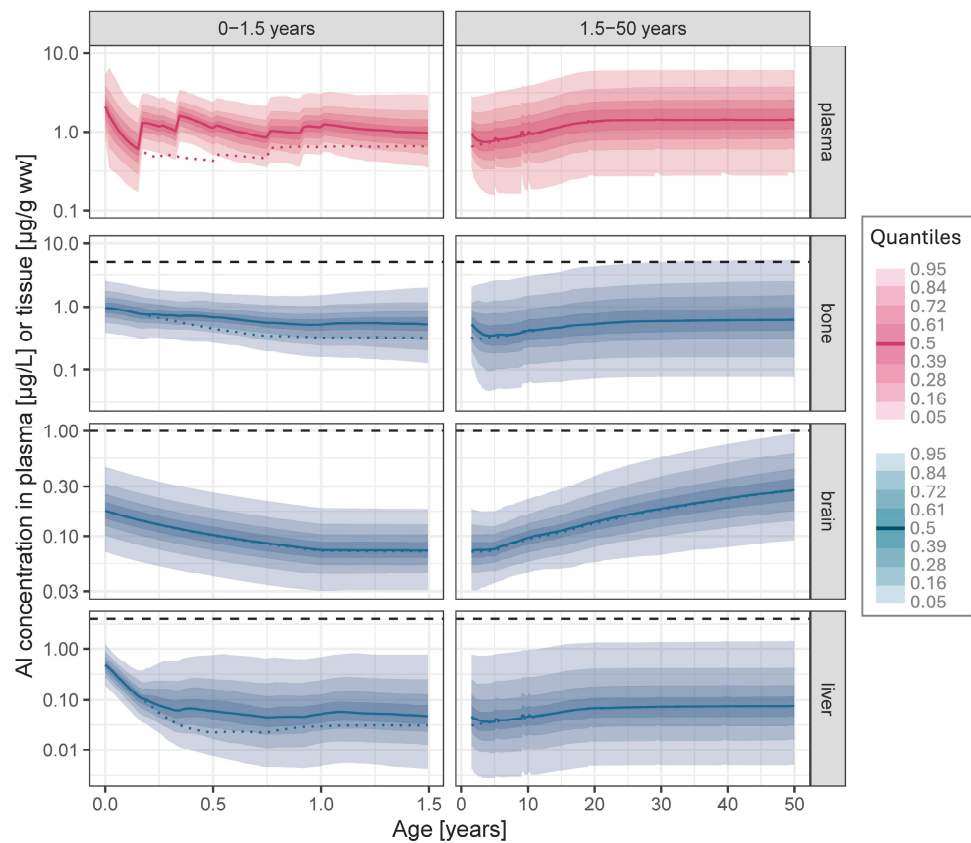


Figure 1. Predicted time courses of Al concentration in plasma, bone, brain, and liver following joint exposure from vaccinations (according to the generally recommended vaccination schedule in Germany 2025 [12], see Table 1) and continuous background dietary intake (food+vacc) from birth to the age of 50 (for better visualization images were divided at age 1.5y; solid line: Median; coloured shaded areas: Quantiles; horizontal dashed line: Upper limit of normal (see Methods); dotted line: Median time course of “food only” exposure).

The main increase in Al levels in plasma and tissues due to vaccinations occurs during the first two years of life with a peak shortly after the vaccinations at 4 months of age (Figure 1, left and Table 2). Median Cmax in plasma reaches 1.6 µg/L (p95: 3.4 µg/L) corresponding to a 3.4-fold increase compared to food only exposure (0.5 (1.5) µg/L; median difference: 1.1 µg/L). A similar increase (2.6-fold; median difference: 0.04 µg/g ww) is observed in the liver. Increase in bone is less pronounced (1.6-fold; 0.2 µg/g ww) and very small in brain (1.03-fold; 0.002 µg/g ww). Despite these increases, in all tissues, maximum Al levels during the first 2 years of life did neither exceed the initial levels at birth nor the ULN in adults (Figure 1). After the vaccinations at the age of 12 months (MenB and MenC) Al levels in plasma and tissues decline slowly and approach baseline (food only exposure) at about 3 (plasma and liver) and 5 (bone) years of age, respectively. As brain is modelled as a sink compartment, the small Al quantities entered into brain persist.

The increase of Al levels due to single (booster) vaccinations in children (at 9y) and adults (at 19y) are hardly visible in plasma or tissues (Figure 1, right). Median ratios (Table 2) indicate a maximum increase over the dietary background exposure of 14% (plasma and liver), 4% (bone), and 3% (brain) in children, and a maximum increase of 4% (plasma), 2% (liver and brain), and 0.4% (bone) in adults.

Table 2. Al concentrations predicted in plasma, bone, brain, and liver following joint exposure from vaccinations (according to the generally recommended vaccination schedule in Germany 2025 [13], see Table 1) and continuous background dietary intake (“food+vacc”, see Figure 1) compared to dietary intake only (“food only”) at the timepoint of the highest median difference between both curves stratified into three age periods (m:

median; p95: 95% quantile; m diff: difference of medians (m(food+vacc)-m(food only)); m ratio: ratio of medians (m(food+vacc)/m(food only)); ww: wet weight).

Age period (years)	Al exposure	Al concentration											
		Plasma (µg/L)			Bone (µg/g ww)			Brain (µg/g ww)			Liver (µg/g ww)		
		m	p95	m diff (m ratio)	m	p95	m diff (m ratio)	m	p95	m diff (m ratio)	m	p95	m diff (m ratio)
Infant (0-2)	food+vacc	1.618	3.433	1.140 (3.39)	0.668	1.579	0.246 (1.58)	0.073	0.179	0.003 (1.04)	0.066	0.713	0.041 (2.64)
	food only	0.478	1.501		0.422	1.146		0.070	0.176		0.025	0.266	
Child (9-10)	food+vacc	1.006	3.733	0.121 (1.14)	0.411	2.944	0.016 (1.04)	0.096	0.227	0.003 (1.03)	0.049	0.934	0.006 (1.14)
	food only	0.885	3.677		0.395	2.860		0.093	0.226		0.043	0.902	
Adult (19-20)	food+vacc	1.380	5.961	0.049 (1.04)	0.533	4.245	0.002 (1.004)	0.136	0.373	0.003 (1.02)	0.068	1.318	0.001 (1.02)
	food only	1.331	5.936		0.531	4.236		0.133	0.366		0.067	1.308	

3.2. Al Exposure from Food and Vaccinations Including “Additional HepB at Birth”

The predictions for the scenario that included additional HepB vaccinations is illustrated in Figure 2. The two additional vaccinations at birth and 1 month lead to a moderate (flat) early rise in plasma and liver Al levels, again less pronounced in bone and negligible in brain. The peak in plasma at 2 months of age is therefore elevated and is now the highest with 1.9 (p95: 4.2) µg/L (Cmax), compared to Cmax of 1.6 (3.4) µg/L at 4 months without additional HepB vaccinations (see Figure 1 and Table 2). In all tissues, the concentrations remain still below the initial levels at birth.

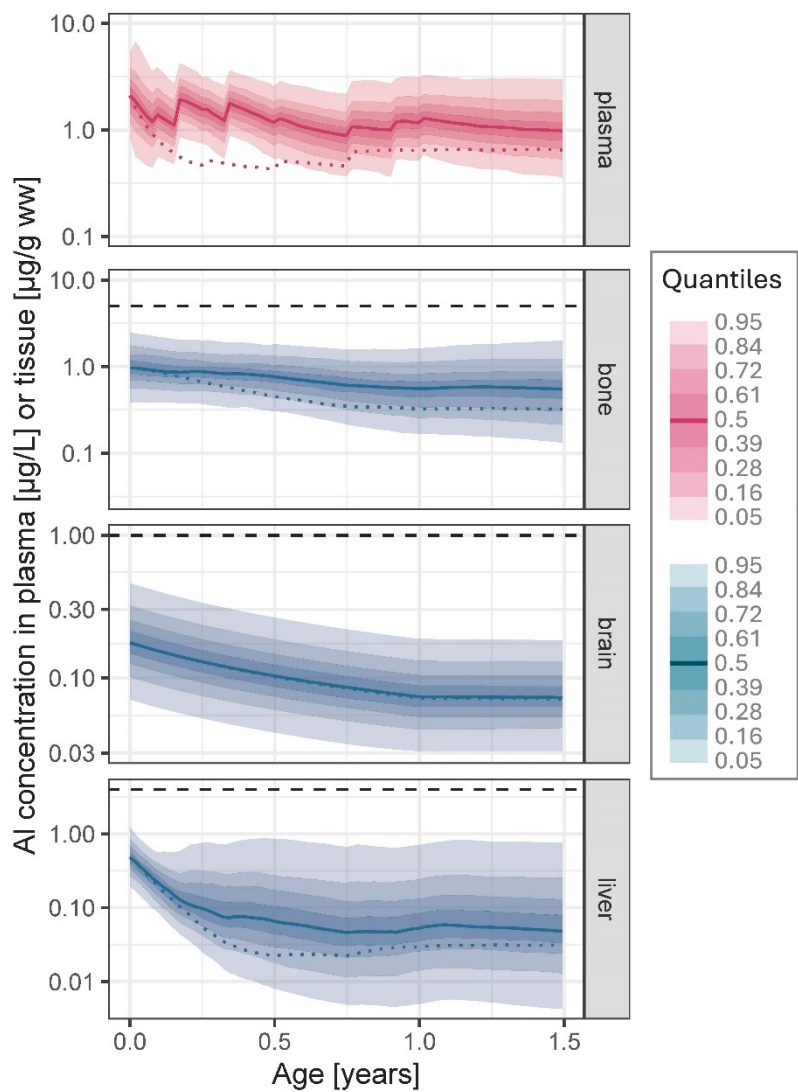


Figure 2. Predicted time courses of Al concentration in plasma, bone, brain, and liver following joint exposure from vaccinations according to the recommended vaccination schedule in Germany 2025 including two additional HepB vaccinations after birth (adjuvant type: AAHS, see Table 1) and continuous background dietary intake from birth to the age of 1.5y (solid line: Median; coloured shaded areas: Quantiles; horizontal dashed line: Upper limit of normal (see Methods); dotted line: Median time course of “food only” exposure).

3.3. Body Burden of Al from Food and Vaccinations after 50 Years

The model-predicted median total amount of Al present in the body of a reference female adult at the age of 50 after continuous dietary intake according to the food only scenario described in 2.1.1. is 5.105 (p5-p95: 0.854-39.280) mg compared to 5.122 (0.862 -39.346) mg for the food+vacc scenario (including “additional HepB at birth”, as described in 2.1.3). This results in a median increase in body burden due to vaccinations after 50 years of life of 17 µg (0.3%).

4. Discussion

These are the first physiologically-based model predictions of concentration-time profiles of Al in plasma and tissues after administration of adjuvanted vaccines in humans on top of an average age-dependent dietary exposure. It is important to emphasize that our predictions are based on a wide range of clinical and physiological data that were used to build and validate the model. All assumptions regarding initial Al levels at birth, physiological growth, Al absorption, and Al bone

kinetics following the human age-dependent Ca turnover have been thoroughly validated [4]. In particular, proof has been shown of the model's qualification for making credible predictions for i.m. administration of Al adjuvanted vaccines in animals [4,25]. Though only sparse data were available for validation of the model performance in infants, the model was considered appropriate for predictions in healthy full-term neonates and infants, as it accounts for the time-based changing physiology with regard to body size, organ volumes and blood flows, including the ontogeny of physiological processes most relevant for Al kinetics, i.e. the maturation of the renal function (GFR), and the age-specific Ca kinetics in bone from birth to adulthood, and for the natal body burden resulting from maternal exposure. Of note is that, due to the model physiology representing the growth of full-term infants (median body weight at birth: 3.5 kg), predictions of Al toxicokinetics in preterm infants are beyond the capabilities of this study.

The predictions demonstrated that single vaccinations in children and adults do not lead to substantial increases of Al concentration neither in blood nor tissues compared to the dietary background level (maximum increase by 14%).

Vaccinations of healthy full-term infants during the first year of life do moderately increase the overall Al exposure. The maximum rise relative to the dietary intake in infants occurs early (at about 4 months of age) showing a 2 to 3-fold increase in Al levels in plasma, liver and bone. However, this increase is transient and occurs in a period when blood and tissue levels decline (from maternal levels) due to the low intake from infant food and dilution by body growth. It was observed early on that Al levels measured in plasma and bone at birth are not different from those known from adults [26]. For example, C_{max} predicted in plasma in vaccinated infants at 4 months (m/p95: 1.6/3.4 µg/L; Table 2) is still within the range of levels measured soon after birth (2-15 µg/L, [4]) or in normal adults (1-10 µg/L; [9]). It is far below Al plasma levels associated with clinical signs of osteomalacia and cholestasis reported from preterm infants on TPN (> 20-30 µg/L; [26–28]). In particular, this is underpinned by the maximum tissue levels predicted in the important target organs bone and liver that also remain below the ULN of adults and far below critical levels described in literature. Most important, the relative increase in Al concentration in brain by 4% due to the vaccinations is minimal. Thus, the main finding of our analysis is that the maximum Al levels predicted in plasma and tissues due to vaccinations in full-term infants during the first year of life do not exceed the levels observed in normal infants soon after birth.

The vaccination schedules in place in the EEA vary [14]. We used the most recent German schedule [13]. The vaccination scheme that includes DTaP ("2 plus 1") is similar to the majority of member states, and represents an early start at 2 months of life. In addition, Al-adsorbed MenC and MenB vaccinations are generally recommended in the first two years of life. Thus, Al exposure from the chosen schedule during the first two years (4.8 mg) is considered representative of other countries. For example, a maximum of 4.5 mg cumulative exposure after 2y was administered in the recent Danish cohort study [7], and for the US, Mitkus et al. [29] calculated 4.2 mg Al based on the US vaccination schedule in 2011.

In some EU countries hepatitis B vaccination at birth is generally recommended. Our simulation revealed that additional HepB vaccinations at birth only marginally increase plasma and tissue levels which still remain below the initial levels encountered at birth. The flat rise of plasma Al is mainly attributed to the low dose (0.25 mg Al) and the slow absorption rate. The rise would have been even more flat in case of use of a product with AH instead of the faster absorbed AAHS.

We are aware that the underlying dietary intake determines the total Al levels reached after vaccinations, and the predicted decline in Al concentrations after birth is mainly attributed to the low infant compared to maternal food intake assumed. The data we used for the continuous dietary Al exposure over age reflect a rational, average European scenario proven to be in line with reference Al tissue levels in literature [4]. However, especially for infants (0-6 months of age) our intake assumption (0.1-0.2 mg/kg/w) is not strictly conservative being a compromise between levels estimated for breast-fed (0.04 mg/kg/w, [12]) and formula-fed average consumer infants (0.21-0.32 mg/kg/w; [8]). The upper bound estimate for high consumers of infant formulas is reported as 0.52

mg/kg/w [8]), and in cases of soy-based or hypoallergenic formulas intake could be as high as 2 mg/kg/w [8,12]. In order to cover worst case assumptions, we investigated the impact of a “maximum infant formula exposure” on our predictions (Figure A1, Appendix). This scenario would also cover the case of lower intake but potentially much higher oral Al bioavailability in infants as compared to adults. As expected, the high 2 mg/kg/w exposure from infant formulas between 0-6 months of life leads to an elevation of Al levels in both curves, however, the maximum total Al levels in plasma and tissues from food and vaccinations are still within the range of initial levels at birth (e.g. p95 in plasma remains <10 µg/L) and far below levels of concern (> 20-30 µg/L, see above). Thus, even in relation to very high and not declining initial levels, the absolute increases predicted (e.g. 1.1 µg/L in plasma) are still considered moderate.

With respect to the first months of life, our predictions for the Al brain uptake could be challenged by the general scientific claim of incomplete blood-brain-barrier (BBB) integrity in early infancy. The BBB represents a multicellular and highly selective permeability barrier system that guarantees brain homeostasis by regulating active transport of nutrients essential for the development of the CNS and provides a defense line against the passage of potentially harmful xenobiotic substances [30,31]. It is present in the majority of brain capillaries and starts forming early during embryogenesis [30]. While the physical barrier of tight junctions can be considered mature at birth the functional activity of various transporters is modulated during the postnatal period [31]. Thus, since BBB is the primary route of brain uptake of metals [32] and Al uptake is suspected to be transporter-mediated (see below), Al transport into brain might be altered during postnatal development.

Al³⁺ ions circulating in plasma are mainly (approx. 90%) bound to the transport protein transferrin (Tf) and secondarily (approx. 10 %) to citrate [33]. The presence of transferrin receptors on brain capillary endothelial cells (BCECs) provides a means by which plasma transferrin-bound iron crosses the BBB [34]. Transferrin receptor (TfR)-mediated transcytosis is also considered a main transfer route of Al³⁺ ions into brain. A second route is a transporter-mediated uptake of Al³⁺ ions bound to citrate (e.g. by the monocarboxylic acid transporter (MCT-1); [32,35]). These transporters might be affected by BBB maturation: For instance, matching the high iron demand during rapid brain growth, TfR expression and iron uptake was found to be 8 times higher in developing (postnatal day 15) than in adult rats (day 70) [34,36]. Vanucci and Simpson [37] found that MCT-1 protein expression in rats peaks during suckling and declined by 50% during maturation indicating a doubling of the brain uptake rate of Al citrate during the first weeks.

Both findings may suggest that, due to BBB immaturity also in human newborns, Al transport into brain could be faster than the rate assumed in our model. However, there are data indicating that there is also efflux of Al citrate through the BBB [32], which would mitigate this effect. In addition, data in juvenile rats are in general considered not predictive, since the rat brain is neurologically very immature at birth compared with humans [31]. Of note is that the brain concentration of 0.3 µg/g ww measured in a child after 5 weeks of TPN in the study of Moreno [38] could be well predicted by our model with the “mature BBB” brain intrusion (uptake) rate [4]. This does not support a significantly elevated Al brain uptake rate during the first weeks of life.

Nevertheless, we investigated the theoretical impact of a unidirectional constant increase in BBB transport of Al during the first six months of life by repeating the simulation of the “food and vaccinations including HepB at birth”-scenario (see Methods) using a 20 times faster intrusion rate for Al from blood into brain while conservatively maintaining the sink situation (rate outwards remained at zero). The resulting Al concentrations in brain would be higher in both scenarios (food only: median (p95): 0.116 (0.296) µg/g ww; food+vacc: 0.139 (0.309) µg/g ww; Figure A2, Appendix). The maximum increase after 6 months due to vaccinations would be 1.2-fold (median difference: 0.024 µg/g ww). The total level would still not exceed the initial level range of infants soon after birth.

Another concern expressed frequently is the contribution of Al from vaccinations to the “body burden” over a lifetime (as a possible cause for neurodegenerative diseases). According to the PBTK model, brain is the only organ where total retention can occur, whereas accumulation in bone is

transient. Due to the continuous age-dependent process of bone remodelling Al (following Ca) is assumed to be slowly but steadily released from bone. The specific bone submodel implemented in the PBTK model reflects these kinetic processes [4,15]. Our calculated body burden of 5 mg at the age of 50 (i.e. amount of Al present in the body at that time) is perfectly in line with Priest et al. [39] who estimated an accumulation of 2-7 mg from oral dietary exposure during adult life based on their ²⁶Al-retention studies. Of note is that this body burden does not include Al particles, e.g. those present in lungs or lymph nodes deriving from inhalative exposure of Al dust, which is however included in higher burden estimates based on extrapolations from chemical analysis of all tissues (35-40 mg; [40]). Most important, our predictions clearly illustrate that the contribution of vaccinations recommended for routine immunization from birth to adulthood to the body burden is negligible.

The latest attempt to predict and assess the risk of Al exposure in infants from vaccinations was done by Mitkus et al. in 2011 [29]. They estimated the total body burden of aluminium retained from dietary and vaccine exposures and compared it to that calculated from the minimal risk levels (MRLs) established by the US Agency for Toxic Substances and Disease Registry (ATSDR) derived from animal studies.

Similarly to our assumptions, they accounted for a background dietary intake and a baseline level of Al in the blood of newborns. In contrast, we additionally based the initial levels at birth also on measurements in other tissues than blood ([4]). To estimate the i.m. absorption rate, Mitkus et al. used data from just 2 rabbits only whereas our estimates were based on additional adjuvant-specific injection site measurements in rats and monkeys [4].

The main advance of our approach, however, is that it allows for a prediction of Al concentrations over time at the tissue level in relevant target organs like bone, brain, and liver, in contrast to their calculations of total Al amounts in the whole body based on an empirical retention function. Furthermore, the physiologically-based approach enables reliable predictions for children and even neonates. Physiological changes with age related to childhood growth and maturation processes (GFR and Calcium oriented Al bone metabolism) are dynamically accounted for.

Trough the use of a toxicokinetic state-of-the-art method, our findings corroborate the previous conclusion drawn by Mitkus et al. [29] that episodic exposures to Al-containing vaccines continue to be extremely low risk to infants.

A recent Danish population-based cohort study provides complementary epidemiological support for this conclusion: Andersson et al. [7] did not find evidence that cumulative Al exposure from vaccination during the first 2 years of life was associated with an increased risk (at least a moderate to strong increase in risk (> 30%)) for any of 50 autoimmune, atopic, and neurodevelopmental disorders assessed (including asthma, atopic dermatitis, rheumatoid arthritis, autism spectrum disorder, and attention deficit-hyperactivity disorder).

Our model-based predictions of maximum Al tissue level increases due to vaccination and these epidemiological results complement each other very well. Our finding of minimal brain Al increases supports the negative findings by Andersson et al. regarding CNS outcome. In addition, we provide supportive evidence that toxicological relevant Al levels are also not to be expected in bone or liver, the toxicological impact of which were not investigated in Andersson's study.

5. Conclusions

In summary, Al concentrations predicted in plasma and tissues after joint exposure of Al from recommended vaccinations with Al-adjuvanted vaccines and continuous dietary background intake from (full-term) birth to adulthood remain far below levels which might be associated with toxicity. Even the moderate increase in Al exposure due to vaccinations during the first two years of life is considered safe, since maximum Al levels predicted in plasma and tissues did not exceed normal levels measured in infants soon after birth or known from adults. Thus, predicted Al increases are in no way commensurate to the life-saving benefit of the vaccinations. We conclude that, even though the cumulative Al amount administered through vaccination has increased over the years, regarding Al toxicity the current recommended vaccination schedules can be considered safe.

The model used in this study has proven a valuable tool for simulations of AI exposure after vaccinations or subcutaneous immune therapy (SCIT; [11]) and may further support risk assessment by regulatory bodies, especially with respect to changing/expanding vaccination and treatment schedules.

Author Contributions: Conceptualization, K.W. and B.K-S.; methodology, G.W. and N.H.; software, N.H. and G.W.; validation, N.H. and W.H.; formal analysis, G.W. and K.W.; writing—original draft preparation, K.W. and G.W.; writing—review and editing, K.W., G.W., N.H., W.H., and B.K-S.; visualization, G.W. and N.H.; supervision, B.K-S; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original data presented in the study are openly available in a publically available repository [<https://doi.org/10.5281/zenodo.18442705>].

Acknowledgments: The authors thank Dr. Walter Matheis (PEI, Infectiology division) for advice and discussion on AI-adjuvant containing vaccine products.

Conflicts of Interest: K.W., G.W., B.K-S., N.H., and W.H. declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AH	Aluminium hydroxide
AP	Aluminium phosphate
AAHS	Amorphous Aluminium hydroxyphosphate sulfate
DTaP-	Diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus
IPV-Hib-	influenzae type b, and hepatitis B combination vaccine (6-in-1)
HepB	
HepB	Hepatitis B Vaccine
HPV	Human Papillomavirus vaccine
Td	Tetanus & diphtheria Vaccine, adult/adolescent formulation
Tdap	Tetanus, diphtheria & acellular pertussis vaccine, adult/adolescent formulation
Tdap-IPV	Diphtheria, tetanus, acellular pertussis and inactivated poliovirus combination vaccine, adult/adolescent formulation
MenB	Serogroup B meningococcal vaccine
MenC	Serogroup C meningococcal vaccine
PCV13/15	Pneumococcal Conjugate Vaccine (13-/15-valent)

Appendix

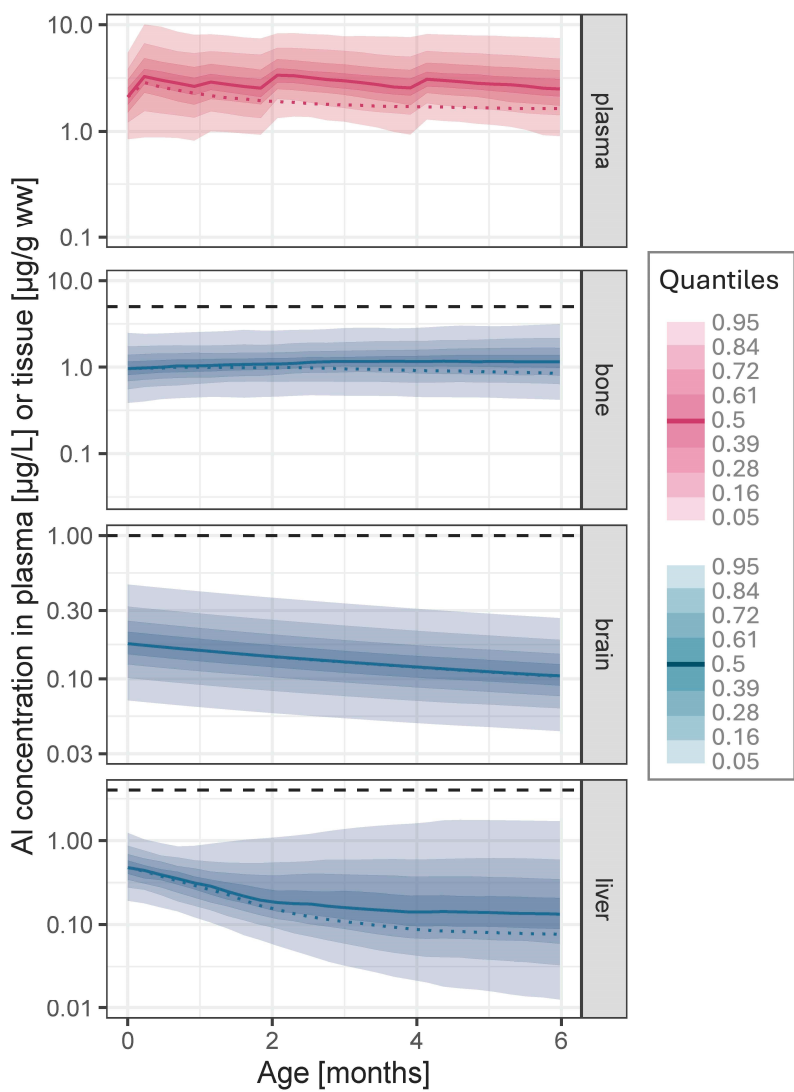


Figure A1. Predicted time courses of Al concentration in plasma, bone, brain, and liver following joint exposure from vaccinations (according to the generally recommended vaccination schedule in Germany 2025 [12] including two additional HepB vaccinations after birth (adjuvant type: AAHS); see Table 1 and Figure 2 in the main text) and continuous background dietary intake (food+vacc) by using a maximum dietary exposure of 2mg/kg/w from birth to the age of 6 months (solid line: Median; coloured shaded areas: Quantiles; horizontal dashed line: Upper limit of normal (see Methods); dotted line: Median time course of "food only" exposure).

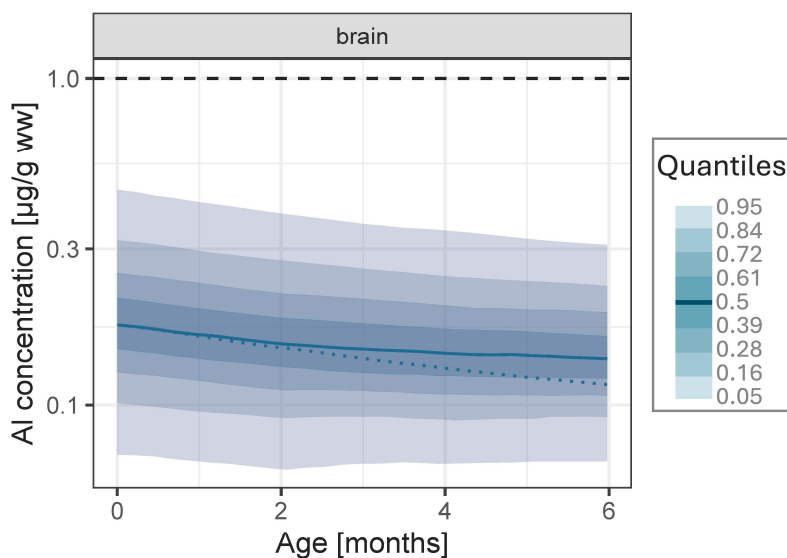


Figure A2. Predicted time courses of Al concentration in brain following joint exposure from vaccinations (according to the generally recommended vaccination schedule in Germany 2025 [12] including two additional HepB vaccinations after birth (adjuvant type: AAHS); see Table 1 and Figure 2 in the main text) and continuous background dietary intake (food+vacc) from birth to the age of 6 months by using a 20 times faster intrusion rate from blood into brain while maintaining the sink situation (rate outwards remained at zero) (solid line: Median; coloured shaded areas: Quantiles; horizontal dashed line: Upper limit of normal (see Methods); dotted line: Median time course of “food only” exposure).

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