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Fluid warming with parylene-coated enFlow cartridge: Bench and pilot animal study of aluminum extraction due to prolonged use

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Abstract

Objectives: Intravenous fluid warming devices with surface heating systems transfer heat using aluminum blocks, which if uncoated elute toxic levels of aluminum into the infusate. This study examined extractable aluminum detected from prolonged use of the updated version of the enFlow® cartridge, which uses a parylene-coated aluminum heating block.

Methods: In dynamic bench tests, we measured the concentration of aluminum that leached into three solutions (Sterofundin ISO, Plasma-Lyte 148, and whole blood) that were continuously pumped (0.2 and 5.5 mL min^{-1}) and warmed to 40°C by the enFlow cartridge (parylene-coated) for 5 h. Prolonged quasi-static bench tests measured aluminum concentration in 16 solutions which were gently rocked within the enFlow cartridge (parylene-coated) for 72 h at 40°C. Aluminum concentrations were measured using inductively coupled mass spectroscopy and matrix blank corrected. Measured aluminum concentrations were compared to a Tolerable Exposure limit to calculate Margins of Safety based on the US Food and Drug Administration maximum recommended concentration in parenteral fluids (25 μg L^{-1}). A parallel pilot in vivo animal study was performed using mice injected with fluids warmed for 72 h by the enFlow cartridge (parylene-coated).

Results: The enFlow cartridge (parylene-coated) demonstrated low toxicological risks in all tests. Sterofundin ISO resulted in the highest aluminum concentration after simulated prolonged use of the enFlow cartridge (parylene-coated) (3.11 μg $device^{-1}$), which represents a 99.2% decrease from the enFlow cartridge (uncoated) and Margin of Safety of 1.7. Dynamic tests at two different flow rates with three challenge solutions resulted in concentrations less than the method detection limits (20.6 or 41.2 μg L^{-1}) of the analysis method. The animals in the in vivo study showed no evidence of toxicity.

Conclusion: Observed toxicological risk levels associated with the enFlow cartridge (parylene-coated) intravenous fluid warmer were below those set by the Food and Drug Administration and suggest that the use of enFlow cartridge (parylene-coated) is safe with a variety of intravenous solution types and in different therapeutic scenarios.

Keywords

Hypothermia, prevention, anesthesia, aluminum toxicity, fluid warming, enFlow

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Introduction

Maintaining a constant body temperature during anesthesia prevents major complications and prolonged hospitalization.[1,2] Therefore, patient-specific temperature management is a major imperative during operative procedures. A drop of body core temperature below 36°C meets the criterion of hypothermia.[3] To help prevent such a decline in core temperature, several intravenous fluid warmers are used in the operating room to warm intravenous fluids. Most warming systems use a disposable cartridge containing a heating block to warm the fluid up to 40°C.[4,5]

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Figure 1. enFlow cartridge (parylene-coated) disposable IV/blood warmer with parylene-coated aluminum warming plate of the patient-contacting disposable cartridge.
Source: Photograph courtesy of Vyaire Medical, Inc., Mettawa, IL, USA.

Some brands of intravenous fluid warmers using aluminum heating blocks have been shown to leach potentially significant amounts of aluminum into the infusate. One group[6] studied a fluid warming device with a coated aluminum heating block (Fluido® Compact, The 37Company, Amersfoort, the Netherlands) and an uncoated device (enFlow® cartridge (uncoated), Vyaire Medical, Inc., Mettawa, IL, USA). The researchers pumped two different infusion solutions (saline and a balanced electrolyte solution) through the two systems for 60 min and evaluated the leached aluminum. They found an increased and potentially unacceptably high level of leached aluminum when using the uncoated system. A second study examined the enFlow cartridge (uncoated) but with blood products as well as an electrolyte product for 60 min.[7] This study confirmed that the enFlow cartridge (uncoated) warmer also leached potentially dangerous levels of aluminum into the warmed intravenous (IV) fluids. Cabrera et al.[8] recently evaluated the aluminum leaching from the Level 1® H-1025 Fast Flow Fluid Warmer (Smiths Medical, Minneapolis, MN, USA). The authors tested three perfusion solutions: saline, Ringer's lactate, and heparinized whole blood, at a constant flow rate of 30 mL min^{-1} over 60 min. They found that the amount of aluminum leached from the system did not reach clinically significant levels, although their findings were subject to debate.[8–10]

The original enFlow cartridge (uncoated) fluid warmer was recently redesigned with a parylene coating on the fluid-contacting portion of the aluminum heating block. This redesigned device, known as the enFlow cartridge (parylene-coated) (Figure 1), is identical to the original enFlow cartridge (uncoated) except for the parylene coating applied to the aluminum heating blocks.

Previous studies evaluated fluid warming devices only for a relatively short time (1 h) using only a few intravenous fluids.[6–8] This study evaluates potential aluminum leaching and its toxicity after a prolonged (5 or 72 h) exposure and for 16 different clinically relevant fluids to simulate a clinical scenario of a patient having multiple surgeries using multiple types of intravenous fluids. We evaluated both lipophilic and hydrophilic fluids with chronic exposures that exceed manufacturers' recommendations. In addition, available literature does not address in vivo correlates of toxicity or biological effects that may arise related to the fluid warmer, so we also performed a pilot in vivo preclinical study in mice using both hydrophilic and lipophilic heated fluids from the enFlow cartridge (parylene-coated). We hypothesized that the coated enFlow cartridge (parylene-coated) system does not result in a significant leaching of aluminum into heated fluids as measured by toxicity assessment.

Methods

We performed three different experiments for this study: dynamic flow fluid analysis, long-term quasi-static fluid analysis, and in vivo animal testing in mice.

Bench testing (dynamic and quasi-static testing)

Two different bench setups and durations were tested: "dynamic" and "quasi-static" (Figure 2). The experimental setup for dynamic testing was similar to previous studies:[6,7] challenge fluids were flowed through the enFlow cartridge (parylene-coated) at a fixed flow rate for 5 h and the outputted fluids were collected. Device warming was activated for the duration of the testing at the fixed temperature of 40°C. Each solution was tested at two different flow rates: 0.2 mL min^{-1} for neonates and 5.5 mL min^{-1} for adults. The aluminum concentration within the outputted fluids was measured and expressed as μg L^{-1}.

For quasi-static testing, methods based on the principles described in ISO 10993-1:2016[11] and ISO 10993-18:2005[12] were followed. Specifically, an enFlow cartridge (parylene-coated) was filled with one of the challenge solutions and capped closed. The cartridges were then placed inside a temperature chamber at 40°C and gently rocked continuously for 72 h. Following 72 h, the total aluminum content within the cartridge was quantified. Since there was no flow during the quasi-static tests, aluminum content was expressed as μg $device^{-1}$.

Quasi-static and dynamic bench testing was managed by Nelson Laboratories, LLC (Salt Lake City, UT, USA) and performed by American West Analytical Laboratories (AWAL, South Salt Lake City, UT, USA). AWAL is accredited by the National Environmental Laboratory Accreditation Program.

Challenge solutions. For dynamic testing, three challenge solutions were examined: Sterofundin ISO (B. Braun Melsungen AG, Melsungen, Germany; Na^+: 145 mmol L^{-1}, K^+: 4 mmol L^{-1}, Ca^{2+}: 2.5 mmol L^{-1}, Mg^{2+}: 1 mmol L^{-1}, Cl^-: 127 mmol L^{-1}, acetate: 24 mmol L^{-1}, and malate: 5 mmol L^{-1}), Plasma-Lyte 148 (Baxter International, Deerfield, IL, USA, Na^+: 140 mmol L^{-1}, K^+: 4 mmol L^{-1}, Cl^-: 98

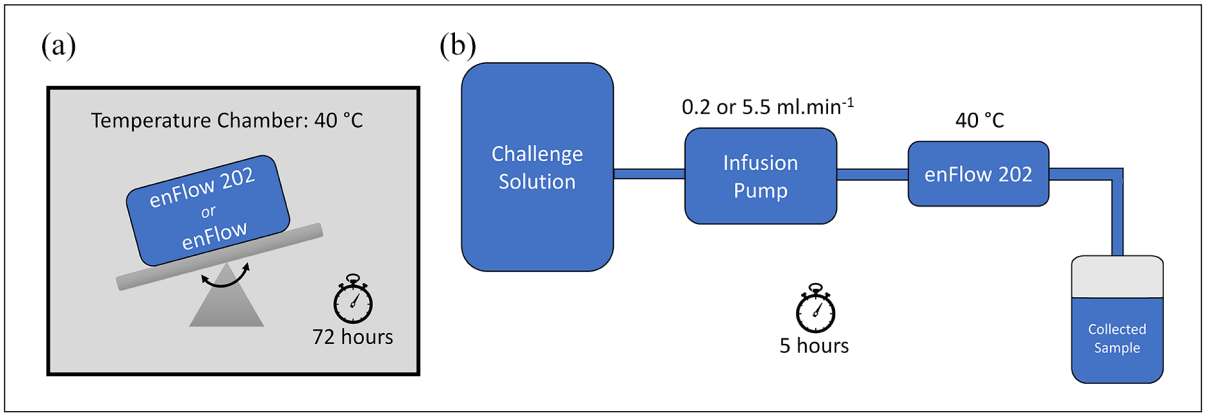

Figure 2. (a) Quasi-static protocol: quasi-static testing, the enFlow cartridge (parylene-coated) was placed in a heated temperature chamber at 40°C and gently rocked for 72 h. (b) Dynamic protocol: dynamic testing at a fixed flow rate for 5 h.

mmol L^{-1}, Mg^{2+} 1.5 mmol L^{-1}, acetate: 27 mmol L^{-1}, and gluconate: 23 mmol.L^{-1}), and whole blood (StemExpress, Folsom, CA, USA). Sterofundin ISO and Plasma-Lyte 148 were studied because previous studies revealed high concentrations of aluminum leached into them when flowed through the enFlow cartridge (uncoated). Whole blood was included because of its common use during surgery.

For quasi-static testing, 16 challenge solutions were tested: Sterofundin ISO (B. Braun); Plasma-Lyte 148 (Baxter International); single donor human whole blood (StemExpress); human packed cells (StemExpress); Ringer's lactate in 5% dextrose (Baxter International; Na^+: 130 mmol L^{-1}, K^+: 4 mmol L^{-1}, Ca^{2+}: 1.4 mmol.L^{-1}, Cl^-: 109 mmol L^{-1}, and lactate: 28 mmol.L^{-1}); human platelet lysate (StemExpress); human buffy coat (StemExpress); human plasma diabetic type 2 (StemExpress); 5% dextrose solution (Pfizer Inc., New York, NY, USA); 3% sodium chloride injection (B. Braun); human serum albumin 25% (StemExpress); normal human serum off-the-clot charcoal-dextran 1 (StemExpress); human cord blood (StemExpress); leukocytes (StemExpress); potassium chloride in 5% dextrose and 0.9% sodium chloride (Pfizer Inc.); and 10% dextrose and 0.45% sodium chloride (B. Braun). Eleven of these 16 challenge solutions are commonly used clinically, and five were included to further test safety and aluminum elution. For the first ten challenge solutions listed above, quasi-static testing was also conducted on the enFlow cartridge (uncoated) for direct comparison with the enFlow cartridge (parylene-coated) results. For dynamic testing, a single run was performed for each of the three challenge solutions and flow rates tested. For quasi-static testing, a single run was done for each of the sixteen challenge solutions with the enFlow cartridge (parylene-coated). Since all bench test results were single data points, no statistical methods were performed.

Analytical chemistry. In both dynamic and quasi-static bench testing, the aluminum concentration post-warming within challenge fluids was determined using inductively coupled mass spectroscopy (ICP/MS). Samples were first digested with a nitric acid (HNO_3) and hydrochloric acid (HCl) mixture. Following preparation, samples were forced through a nebulizer and converted into an aerosol. The resultant aerosol is then forced through a plasma which ionizes the atoms. The ionized atoms are extracted from the plasma by a vacuum interface and directed through a quadrupole which separates the ions by mass-to-charge ratio.

The aluminum preparation and analyses for each sample of challenge fluids used matrix spikes, matrix spike duplicates, and matrix blanks in addition to the typical analytical laboratory quality control samples. Matrix blanks followed the same procedure and analysis as matrix samples but were not incubated. Using these additional quality control samples allows for evaluation of matrix effects on the detection of aluminum. Since the matrices used may have had inherent aluminum, all results were matrix blank corrected to determine device-related extractable aluminum amounts.

The method detection limit is defined by the US Environmental Protection Agency (EPA)[13] as the minimum concentration a substance can be measured with 99% confidence that the concentration is greater than zero. The method detection limit is dependent on the instrumentation, matrix, and skill of the operator. The reporting limit, also known as the practical quantitation limit, represents the smallest concentration of aluminum that can be detected within a sample and can be reported with a reasonable degree of accuracy. The reporting limit is typically two to five times larger than the method detection limit.

For the dynamic tests, aluminum concentration method detection limits were 20.6 μg L^{-1} for Sterofundin ISO and Plasma-Lyte 148 solutions and 41.2 μg L^{-1} for whole blood. The reporting limits were 50 μg L^{-1} for Sterofundin ISO and Plasma-Lyte 148 solutions and 100 μg L^{-1} for whole blood. For quasi-static test, aluminum concentrations and reporting limits were expressed as μg $device^{-1}$. For most solutions, approximately 5 mL of matrix was recovered from the cartridge after warming. Therefore, a reporting limit of 50 μg L^{-1} is equivalent to 0.250 μg $device^{-1}$. Aluminum concentrations reported by the analytical methods used are within 10%

of the true value based on the quality control requirement of the laboratory.

Establishing acceptance criteria for bench testing. To determine the toxicological hazard of the enFlow cartridge (parylene-coated), we compared the measured aluminum concentrations to the Tolerable Exposure (TE) levels for aluminum estimated based on guidelines described in ISO 10993-17:2002.[14] TE levels represent the maximum dose at which exposure to the substance does not produce adverse events or pose an unacceptable risk to human health.[15] In this study, we estimated the worse-case TE as a chronic exposure beyond 24 h in neonatal populations. Specifically, TE was defined based on the FDA's recommended maximum concentration of aluminum for large volume parenteral products (25 μg L^{-1}).[16] For the quasi-static bench tests, the total aluminum leached into the fluid within the cartridge was quantified over the 72-h duration. We therefore calculated the minimum TE assuming the lowest parenteral nutrition volume (0.060 L kg^{-1} day^{-1}) for the standard infant weight specified in ISO 10993-17:2002 (3.5 kg)[14]

$$\mathrm{TE} = 25\,\mu\mathrm{g\,L^{-1}} \times 0.060\,\mathrm{L\,kg^{-1}\,day^{-1}} \times 3.5\,\mathrm{kg} = 5.25\,\mu\mathrm{g\,day^{-1}}$$

To characterize the hazard associated with each substance, Margin of Safety was quantified as the ratio of TE to the measured aluminum concentration

$$\text{Margin of Safety} = \frac{\mathrm{TE}}{\text{Measured aluminum concentration}}$$

Margin of Safety is a unit-less index which indicates a fold-level difference between the threshold and measured exposure level. A Margin of Safety greater than 1.0 indicates low toxicological risk.[14] The worst-case Margin of Safety was calculated as the ratio between the TE and the challenge solution with the highest concentration of aluminum leached from the enFlow cartridge (parylene-coated). To calculate Margin of Safety, the aluminum content that accumulated within the enFlow cartridge (parylene-coated) over the 72-h period was compared to a 24-h TE.

For the challenge solutions which were tested with both the enFlow cartridge (uncoated) and the enFlow cartridge (parylene-coated), the percent decrease in the measured aluminum concentration when using the enFlow cartridge (uncoated) to the enFlow cartridge (parylene-coated) was quantified for each solution

$$\text{Percent decrease} = \frac{\text{Concentration}_{\text{enFlow cartridge (uncoated)}} - \text{Concentration}_{\text{enFlow cartridge (parylene-coated)}}}{\text{Concentration}_{\text{enFlow cartridge (uncoated)}}} \times 100\%$$

In vivo animal testing in mice. In addition to the dynamic and quasi-static bench testing, a pilot preclinical acute systemic toxicity testing was conducted on mice using the enFlow cartridge (parylene-coated) to assess the potential health hazards associated with acute exposure using the warmed fluids from the device. Animal testing was managed by Nelson Laboratories, LLC and performed by American Preclinical Services, LLC (Minneapolis, MN, USA) in compliance with ISO 10993-12:2012,[15] ISO 10993-11:2017,[17] and FDA Good Laboratory Practice 21 CFR Part 58.[18] The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of American Preclinical Services, LLC (APS Study ID: PRF922-ST10). A total of 20 male albino outbred strain mice (10 test mice and 10 negative control mice) were used in the study. Use of the enFlow cartridge (parylene-coated) was simulated by injecting the mice with a solution (saline or sesame seed oil) that was previously heated and agitated inside an enFlow cartridge (parylene-coated). The test extracts were prepared according to ISO 10993-12:2012.[15] Specifically, a total solution volume of 73.6 mL was used based on an extract ratio of 3 cm^2 mL^{-1} and total enFlow cartridge (parylene-coated) surface area of 220.8 cm^2. An enFlow cartridge (parylene-coated) was filled with the solution and submerged in the remaining volume and then continuously agitated on an orbital shaker at 60 r/min at 50°C for 72 h. The solutions were then extracted from the cartridges and injected into the test mice within 24 h without alteration. The concentration of Al in the extract was not quantified. Preparation of control extracts was identical to test extracts but without the enFlow cartridge (parylene-coated). Test mice received a single 50 mL kg^{-1} injection of the test extract and control mice received a single 50 mL kg^{-1} injection of control extract on Day 0. For each group, five mice received extracts using normal saline via IV injection and five received extracts using sesame seed oil via intraperitoneal injection. Animal bodyweight was measured immediately prior to the injection (Day 0) and then daily for the next 3 days. Overall, animal health and signs of acute toxicity were monitored at 4 ± 0.25 h, 24 ± 2 h, 48 ± 2 h, and 72 ± 2 h post-injection by comprehensive clinical observations by trained personnel. Specifically, the following were monitored: changes in skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic, and central nervous systems; and somatomotor activity and behavior patterns.

Data and statistical analyses. Mean values and standard deviations of the animal weights for the control and test groups were quantified for each injection solution at each measurement time. Percent change in bodyweight from time 0–72 h

post-injection was calculated for each animal. Two success criteria were defined prior to the start of the preclinical study. The first success criterion was that no animals in each five-animal test group showed greater biological reactivity during the 3-day observation period. The second success criterion was that all the following were met for each five-animal test group: less than two animals died; less than two animals experienced convulsions or prostration; and final bodyweight changed by less than 10% in less than three animals.

This in vivo study was a first-in-animal pilot study. Therefore, no a priori sample size justification was performed and five animals per group were chosen to provide pilot data for future studies.

Independent and dependent variables. For the bench testing, the independent variables were protocol type (i.e. dynamic vs quasi-static), challenge solution, and flow rate. The dependent variable was measured aluminum concentration. To understand the hazard associated with each substance, we calculated Margins of Safety by comparing these measured aluminum concentrations to TE limits. For the in vivo testing, we compared the physiological responses of mice that received injections simulating the use of the enFlow cartridge (parylene-coated) to control injections without the enFlow cartridge (parylene-coated). We tested both intravenous and intraperitoneal injections. The dependent variables were animal bodyweight, overall animal health, and signs of acute toxicity at 4, 24, 48, and 72 h post-injection. All data analyses were performed using MATLAB (MathWorks, Natick, MA, USA).

Results

Dynamic bench testing

The concentration of aluminum in the solutions following dynamic testing using the coated enFlow cartridge (parylene-coated) was less than the method detection limit for both flow rates and all three solutions. Specifically, the concentrations were less than 20.6 μg L^{-1} for Sterofundin ISO and Plasma-Lyte 148 solutions and less than 41.2 μg L^{-1} for whole blood.

Quasi-static bench testing

For quasi-static testing of the enFlow cartridge (parylene-coated), the derived Margin of Safety values for aluminum were above a value of 1.0. Table 1 shows the uncorrected, matrix blank, and blank corrected aluminum concentrations for the 16 challenge IV solutions. Blank corrected aluminum concentrations represent the aluminum added to the solution from the enFlow cartridge and were calculated by subtracting the matrix blank concentration from the uncorrected aluminum concentration. The method detection limit and reporting limits are also tabulated and varied between the different challenge solutions. The reporting limits for single donor human whole blood, 5% dextrose solution, and 3% sodium chloride injection USP were raised due to sample matrix interferences. Note that blank corrected concentrations can be less than the reporting limits because it is calculated by subtracting the matrix blank from the uncorrected concentration. Table 2 compares the amount of aluminum detected in the 16 challenge IV solutions heated at 40°C (104°F) for 72 h with the enFlow cartridge (uncoated) and enFlow cartridge (parylene-coated). For the 10 challenge solutions that were tested with both the enFlow cartridge (uncoated) and coated enFlow cartridge (parylene-coated), the aluminum concentration decreased by at least 98.9% for all solutions except for 3% sodium chloride injection USP (36.4% decrease). Margin of Safety estimates for the enFlow cartridge (parylene-coated) based on a TE of 5.25 μg $device^{-1}$ are also included in Table 2 for each of the challenge solutions. The aluminum content for the most commonly used fluids in clinical practice was 0.090 μg $device^{-1}$ (human packed cells), 0.731 μg $device^{-1}$ (human platelet lysate), 0.833 μg $device^{-1}$ (single donor human whole blood), 1.32 μg $device^{-1}$ (Plasma-Lyte 148), 2.62 μg $device^{-1}$ (Ringer's lactate in 5% dextrose), and 3.11 μg $device^{-1}$ (Sterofundin ISO). The highest aluminum content for all challenge solutions tested was in Sterofundin ISO. The aluminum content that leached into the Sterofundin ISO using the enFlow cartridge (parylene-coated) (3.11 μg $device^{-1}$) represents a 99.2% decrease compared to aluminum content leached when using the enFlow cartridge (uncoated) (376 μg $device^{-1}$). The Margin of Safety for the enFlow cartridge (parylene-coated) when using Sterofundin ISO is 1.7. The total volume of Sterofundin ISO extracted from the enFlow cartridge (parylene–coated) after the 72-h incubation period was approximately 5 mL. Therefore, the final concentration of aluminum that accumulated over 72 h was approximately 622 μg L^{-1}.

For both dynamic and quasi-static bench testing, results for the laboratory control samples, laboratory control sample duplicates, matrix spikes, and matrix spike duplicates were all within the quality control limits for percent recovered and relative percent difference.

In vivo animal study

All 20 animals survived the preclinical testing and were in overall good health over the course of the study. Test animals, which received injections simulating use of the enFlow cartridge (parylene-coated), showed no greater reaction to the injection compared to the control animals. The animals weighed between 25.5 and 34.4 g at the start of the study and none developed weight loss greater than 10% over the course of the study (Table 3).

Discussion

This study found that the enFlow cartridge (parylene-coated), when used in both acute and chronic exposures, resulted in

Table 1. Uncorrected, matrix blank, and blank corrected aluminum concentrations from solutions heated at 40°C (104°F) for 72 h with the enFlow cartridge (parylene-coated). Blank corrected aluminum concentrations represent the aluminum added to the solution from the enFlow cartridge and were calculated by subtracting the matrix blank concentration from the uncorrected aluminum concentration. Method detection limit and reporting limits varied between the different challenge solutions. The reporting limits for single donor human whole blood, 5% dextrose solution, and 3% sodium chloride injection USP were raised due to sample matrix interferences. Results are sorted from the lowest to highest enFlow cartridge (parylene-coated) aluminum concentration.

Solution	Method detection limit ($\mu g\ device^{-1}$)	Reporting limit ($\mu g\ device^{-1}$)	enFlow cartridge (parylene-coated) uncorrected ($\mu g\ device^{-1}$)	enFlow cartridge (parylene-coated) matrix blank ($\mu g\ device^{-1}$)	enFlow cartridge (parylene-coated) blank corrected ($\mu g\ device^{-1}$)
Human serum albumin 25%, 100 mL*	0.206	0.500	<0.500	<0.500	<0.500
Single donor human whole blood*	0.343	0.833	<0.833	<2.50	<0.833
Normal human serum off-the-clot, charcoal-dextran	0.206	0.500	1.700	1.680	0.020
Leukocytes*	0.0412	0.100	0.381	0.336	0.045
Human buffy coat	0.0412	0.100	0.413	0.368	0.045
Human cord blood	0.0412	0.100	0.746	0.666	0.080
Human packed cells*	1.03	2.50	3.010	2.920	0.090
Human plasma, diabetic type 2	0.206	0.500	0.444	0.319	0.125
5% dextrose solution*	0.103	0.250	0.133	<0.250	0.133
Human platelet lysate*	0.206	0.500	1.120	0.389	0.731
3% sodium chloride injection USP*	0.103	0.250	0.897	<0.250	0.897
Potassium Cl in 5% dextrose and 0.9% sodium chloride*	0.206	0.500	1.10	<0.500	1.11
10% dextrose and 0.45% sodium chloride	0.206	0.500	1.11	<0.500	1.11
Plasma-Lyte 148*	0.206	0.500	1.32	<0.500	1.32
Ringer's lactate in 5% dextrose*	0.206	0.500	2.62	<0.500	2.62
Sterofundin ISO*	0.103	0.250	3.11	<0.250	3.11

Solutions marked with an asterisk (*) are commonly used in clinical practice.

Table 2. Quantitatively measured aluminum from solutions heated at 40°C (104°F) for 72 h with the enFlow cartridge (uncoated) and enFlow cartridge (parylene-coated). As the challenge solutions may have inherent aluminum, results were matrix blank corrected to determine device-related extractable aluminum amounts. Results are sorted from lowest to highest enFlow cartridge (parylene-coated) aluminum concentration. Margins of Safety for the enFlow cartridge (parylene-coated) were calculated based on a Tolerable Exposure (TE) of 5.25 $\mu g\,day^{-1}$ which is derived from the FDA aluminum concentration limit for parenteral fluids of 25 $\mu g\,L^{-1}$.

Solution	enFlow cartridge (uncoated) blank corrected ($\mu g.device^{-1}$)	enFlow cartridge (parylene-coated) blank corrected ($\mu g.device^{-1}$)	Percent decrease vs enFlow cartridge (uncoated)	enFlow cartridge (parylene-coated) Margin of Safety
Human serum albumin 25%, 100 mL*	–	<0.500	NA	10.5
Single donor human whole blood*	392	<0.833	>99.8%	6.3
Normal human serum off-the-clot, charcoal-dextran	–	0.020	NA	263
Leukocytes*	–	0.045	NA	117
Human buffy coat	158	0.045	100.0%	117
Human cord blood	–	0.080	NA	65.6
Human packed cells*	113	0.090	99.9%	58.3
Human plasma, diabetic type 2	1310	0.125	100.0%	42.0
5% dextrose solution*	12.1	0.133	98.9%	39.5
Human platelet lysate*	1290	0.731	99.9%	7.2
3% sodium chloride injection USP*	1.41	0.897	36.4%	5.9
Potassium Cl in 5% dextrose and 0.9% sodium chloride*	–	1.10	NA	4.8
10% dextrose and 0.45% sodium chloride	–	1.11	NA	4.7
Plasma-Lyte 148*	4.860	1.32	100.0%	4.0
Ringer's lactate in 5% dextrose*	479	2.62	99.5%	2.0
Sterofundin ISO*	376	3.11	99.2%	1.7

NA: not applicable.
Solutions marked with an asterisk (*) are commonly used in clinical practice.

Table 3. Bodyweight of control and test animals given normal saline and sesame seed oil injections. Bodyweights measured immediately before injection (Day 0) and 72 h later are listed. The percent change from Day 0 to 72 h was well less than 10% for all animals. Data are listed as mean value (standard deviation).

Bodyweight				
Injection	Group	Day 0 (g)	72 h (g)	Percent change (%)
Normal saline	Control	30.3 (1.5)	30.4 (1.2)	0.5 (1.5)
	Test	30.5 (1.1)	30.3 (1.6)	−0.6 (1.9)
Sesame seed oil	Control	30.6 (1.7)	30.4 (1.6)	−0.5 (2.1)
	Test	30.0 (3.4)	30.0 (3.4)	0.0 (1.1)

minimal aluminum elution and favorable derived Margin of Safety above values of 1.0, correlating with safe patient exposure levels that are below those set by the FDA.[16,19] Dynamic tests at two different flow rates with three challenge solutions resulted in concentrations less than the method detection limits (20.6 or 41.2 μg L^{-1}) of the analysis method, levels comparable with other marketed warming devices. This result is consistent with the finding from a previous study conducted on a different coated IV fluid warmer.[6] In that study, the exposure was limited to 1 h compared to 5 h in this study.

The two flow rates tested in the dynamic bench testing were chosen to simulate the range of typical clinical conditions. Specifically, the 0.2 mL min^{-1} is a typical rate for maintenance fluids in a neonate. Standard practice dictates 4 mL $kg^{-1} h^{-1}$ for the first 10 kg in body weight. For a 3-kg neonate, this equates to 0.2 mL min^{-1}. The 5.5-mL min^{-1} flow rate was selected to represent an adult patient undergoing a major surgery under anesthesia. For adults, standard practice recommends 4 mL h^{-1} kg^{-1} for the first 10 kg, 2 mL kg^{-1} for the second 10 kg, and 1 mL kg^{-1} for the remaining body weight. For a 70-kg patient, this corresponds to 110 mL h^{-1}. We further assume the patient has fasted for 8 h (i.e. 880 mL) which will be replaced evenly over a 4-h surgery (i.e. 220 mL h^{-1}). Therefore, 330 mL h^{-1} (i.e. 5.5 mL min^{-1}) was used to represent a typical adult infusion rate.

The quasi-static bench testing enabled us to simulate prolonged use of the enFlow fluid warming system. We quantified the total aluminum that leached into the enFlow cartridge over a 72-h period while the cartridge was gently rocked in a 40°C temperature chamber. Since we only quantified the aluminum at the end of the 72-h period, we do not know if the aluminum leached at a continuous rate over this period. It is plausible that the rate of leaching was the highest at the beginning of the incubation period and then was lower for the remaining time. We therefore decided to calculate Margin of Safety by comparing the 3-day aluminum content to a single-day TE threshold. This calculation assumes that the total amount of aluminum was extracted within the first 24 h.

The highest concentration of aluminum for the quasi-static testing was 3.11 μg $device^{-1}$ with Sterofundin ISO. Since there was approximately 5 mL of solutions within the device, the final concentration of aluminum within the solution was approximately 622 μg L^{-1}. However, it is unlikely that this amount of aluminum would leach into the challenge solution while it is flowing through the device during clinical use. The dynamic bench test presented in this study showed aluminum concentrations are $<$20.6 μg L^{-1} for Sterofundin ISO at both 0.2 and 5.5 mL/min flow rates. Furthermore, previous studies have shown the chemical reactions between the aluminum ions and solutions are rate limited. Specifically, the maximum aluminum concentrations that leached into Plasma-Lyte 148 from the enFlow cartridge (uncoated) was an order of magnitude lower at a high flow rate (16.6 mL min^{-1} and 658 μg L^{-1}) compared to a low flow rate (2 mL min^{-1} and 6028 μg L^{-1}).[7] In addition, the amount of aluminum that leached into the solution increased over the period of an hour. While the mechanisms by which aluminum binds to a challenge solution are unknown, one hypothesis is that the aluminum ions form ionic complexes through carboxyl groups of certain organic anions such as acetate within balanced salt solutions.[20] This hypothesis could explain the large variation in the amount of aluminum that leached into the different challenge solutions.

We estimated TE based on the FDA recommendation of 25 μg L^{-1} for large volume parenteral injection. To achieve the most conservative estimate of TE, we used the flow rate of 60 mL $kg^{-1} day^{-1}$ (i.e. 210 mL day^{-1} for a 3.5-kg infant) which represents the minimum flow rate typically used for parenteral nutrition. The same FDA standard also recommends a maximum parenteral Al level of 4–5 μg $kg^{-1} day^{-1}$ for a patient with impaired kidney function.[21,22] For a 3.5-kg infant, this limit corresponds to a TE of 14 μg day^{-1}, which is a higher threshold than the 5.25 μg day^{-1} we selected.

In clinical use, IV fluid and blood warmers are used with a wide range of solutions such as saline and electrolyte solutions as well as blood and blood products. Each solution has unique thermochemical properties and interaction with the warmer's cartridge and may therefore result in different amounts of aluminum leaching into the solution. The previous studies mentioned above examined aluminum exposure when using IV fluid warmers with a small subset of potential solutions. This study expanded on these studies and measured aluminum elution after prolonged exposure to 16 different clinically relevant challenge fluids when using the enFlow cartridge (parylene-coated). For 10 of these 16

fluids, the aluminum exposure was also tested using the enFlow cartridge (uncoated) and compared to the exposure when using the enFlow cartridge (parylene-coated).

Recent bench testing from other laboratories revealed potentially unsafe levels of aluminum leaching into solutions from the enFlow cartridge (uncoated).[6,7] Specifically, Perl et al.[6] found an aluminum concentration of approximately 6000 μg L^{-1} when flowing Sterofundin through an enFlow cartridge (uncoated). Taylor et al. expanded upon this study, examining aluminum concentrations for a total of five solutions. They found levels of aluminum exposure in Plasma-Lyte 148 and compound sodium lactate solutions that were comparable to those found in Sterofundin in Taylor et al.[7] The results of this study confirmed that the parylene coating on the cartridge significantly reduced the amount of leaching of aluminum into a wide range of clinically relevant challenge solutions (see Table 2).

There are three significant strengths of this research. First is that we tested the enFlow cartridge (parylene-coated) for prolonged exposure to fluids. In general, fluid warmers in an individual patient could potentially be required for time intervals of 1–12 h, depending on the extent of the surgery and resuscitative efforts. Difficult individual cases may then require repeated surgeries with renewed need for fluid warming. Because of this, we chose to study the effects of 72 h of exposure to enFlow, analogous to 14 5-h surgeries. Second, most studies have only addressed a few representative fluids, limiting the generalizability of their research. We sought to evaluate the safety of this device over a much broader spectrum of fluids that might actually be used in direct patient care. We chose 16 different IV solutions that were clinically relevant in anesthesiology from simple (saline) to complex (whole blood), encompassing lipophilic (such as whole blood, platelet lysate, and buffy coat) and hydrophilic (such as Sterofundin, saline, and dextrose in water), and found no evidence of aluminum leaching into the fluid using enFlow cartridge (parylene-coated). Finally, most published studies lack in vivo assessments of aluminum levels resulting from fluid warmers. Therefore, we added the studies on mice to evaluate the potential impact of aluminum toxicity resulting from the use of the enFlow cartridge (parylene-coated). This study was specifically designed to address the major concerns of practicing clinicians.

A limitation of this study is that the aluminum concentration minimum detection limit of our analysis method was 41.2 μg L^{-1} for whole blood. Therefore, in the dynamic testing using whole blood, it was not possible to differentiate between the measured aluminum concentration and the most stringent FDA standard of 25 μg L^{-1}. Regardless, the results of quasi-static testing show aluminum concentrations under this threshold for all challenge solutions. We did not compare enFlow cartridge (parylene-coated) to devices other than the original enFlow cartridge (uncoated) using the same experimental setup, so no assertions can be made about comparisons between other brands of devices. As mentioned, our test methods, while standard, were different from those previously used with other warming devices, making direct comparisons to other studies impossible. To determine our TE, we used the standard infant weight (3.5 kg) specified in ISO 10993-17:2012[15] which exceeds values typical of premature neonates (e.g. 500 g to 2.5 kg).

The in vivo testing in mice provides a first-in-animal pilot study of acute systemic toxicity exploring the potential health hazards associated with use of the enFlow cartridge (parylene-coated) following the procedures described in ISO 10993-11:2017[17] and ISO 10993-12:2012.[15] All animals in the study survived, remained in overall good health, and showed no weight loss. The preclinical study had several limitations which should be addressed in future studies. First, this study only tested saline and sesame seed oil solutions, while future studies should use a balance salt solution such as Sterofundin ISO since our bench testing has shown it results in the highest concentration of aluminum leaching. In addition, future studies should measure the concentration of aluminum within the injected extract to quantify the aluminum dose. Finally, preclinical study in large animals would enable simulated use of the device at clinically relevant flow rates as well as quantification of the change in blood plasma aluminum concentration of the animal resulting from the infusion.

Conclusion

The results of these experiments indicate that observed toxicological risk levels associated with the enFlow cartridge (parylene-coated) intravenous fluid warmer were below those set by the FDA and other regulatory bodies and suggest that the use of enFlow cartridge (parylene-coated) is safe with a variety of IV solution types and in different therapeutic scenarios. The enFlow cartridge (parylene-coated) showed marked improvement in safety compared to its predecessor, the enFlow cartridge (uncoated).

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Animal welfare

This study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

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