Saturable Tissue Binding and Imirestat Pharmacokinetics in Rats

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To investigate the hypothesis that the pharmacokinetics of imirestat, an aldose reductase inhibitor, are influenced by saturable binding to tissues, three experiments were done. (1) The nature of the dose dependence was characterized in rats. Two groups of nine adult male Sprague–Dawley rats received iv ^{14}C-imirestat at doses of 2 or 8 mg/kg. Serial blood samples were obtained over 15 days. Volume of distribution at steady-state was significantly different between the high- and the low-dose groups (0.744 ± 0.103 l and 1.10 ± 0.228 L, respectively). Clearance was independent of dose over this fourfold range (~15 ml/hr). (2) The effect of either statil or AL3152, both aldose reductase inhibitors and potential competitors for aldose reductase binding, on the pharmacokinetics of a single 0.2-mg/kg iv dose of imirestat was assessed. A 2.4-mg/kg loading dose of statil was administered and a constant-rate infusion (56 μg/hr/kg) was begun 16 hr before imirestat. A 2-mg/kg loading dose of AL3152 and a constant-rate infusion (115 μg/kg/hr) were also administered 16 hr before imirestat. The infusions were maintained throughout the study. AL3152 administration decreased the imirestat steady-state volume of distribution by a mean of 63%. Statil administration decreased it by a mean of 39%. (3) The dosing regimen of the second study was repeated and, at two sampling times, nine tissues and plasma were obtained from four rats per sampling time for determination of imirestat tissue-to-plasma concentration ratio. The tissue/plasma imirestat concentration ratio in the adrenals 24 hr after imirestat administration was 56.9 ± 20.0 in the imirestat group, 17.7 ± 1.27 in the statil-coadministered group, and 12.3 ± 2.59 in the AL3152-coadministered group. A similar trend of decrease in the ratios was observed in all tissues at both 24 and 168 hr. The results suggest that a saturable tissue binding phenomenon at least partially accounts for the nonlinear pharmacokinetics of imirestat.

KEY WORDS: imirestat; statil; pharmacokinetics; tissue binding.

INTRODUCTION

Aldose reductase inhibitors have the potential to limit the complications of diabetes which develop in noninsulin-sensitive tissues. Among the possibly preventable complications are peripheral neuropathy, retinopathy, and cataract. Damage in these tissues is thought to be osmotic, resulting from excessive accumulation of sorbitol produced from glucose via aldose reductase (1–5).

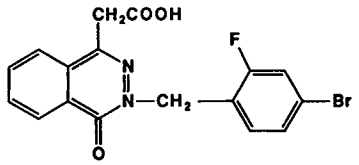
Imirestat (Fig. 1) is one of a series of potent spirohydantoin aldose reductase inhibitors (6,7). As expected from its structure, it is a poor substrate for hepatic drug metabolizing enzymes and is handled inefficiently by renal mechanisms of elimination. In humans, the drug has an extremely long half-life (>200 hr) at a dose of 2 mg, which diminishes to 60–70 hr as the dose is increased to 50 mg. Clearance is apparently independent of dose and is low, 30–40 ml/min (8).

In rats receiving a single iv dose of 4 mg/kg, the apparent half-life of imirestat is 30 hr (9), but the tissue/blood ratio of drug concentration increases substantially between 24 and 168 hr, while the blood/plasma concentration ratio is relatively constant (10). For example, the tissue/blood concentration ratio increases 2.5- to 3.5-fold in kidneys, adrenals, and heart over this period; 7-fold in lungs; 10-fold in testes; and 13.5-fold in eyes. The persistence of imirestat in rat tissues and the decrease in apparent half-life with increasing dose suggest that the pharmacokinetics of the drug may, in part, be influenced by a saturable tissue binding phenomenon. We have conducted three studies to evaluate this hypothesis: (i) a detailed study of the dose dependence of the pharmacokinetics of imirestat in rats, (ii) a study of the effect of administration of two other structurally distinct aldose reductase inhibitors of comparable potency (6,7,11) (AL3152 and statil; Fig. 1) on the pharmacokinetics of imirestat, and (iii) the effect of the other aldose reductase inhibitors on the tissue/plasma concentration ratio of imirestat.

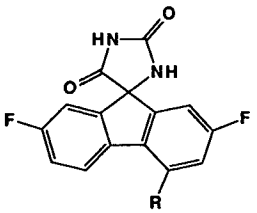
MATERIALS AND METHODS

Materials

Imirestat [2,7-difluoro-spiro (9H-fluorene-9,4′-imidazolidine)-2′,5′-dione] and AL3152 [2,7-difluoro-4-methoxyspiro (9H-fluorene-9,4′-imidazolidine)-2′,5′-dione] were obtained from Alcon Laboratories, Fort Worth, TX. Statil [3-(4-bromo-2-fluorobenzyl)-4-oxo-3H-phthalazine-1-xylacetic acid] was obtained from ICI Pharmaceutical Division, Cheshire, England. [15′-^{14}C]Imirestat was obtained from Pathfinder Laboratories, Inc. (St. Louis, MO), had a



STATIL



IMIRESTAT R = H
AL3152 R = OCH_3

Fig. 1. Structures of aldose reductase inhibitors.

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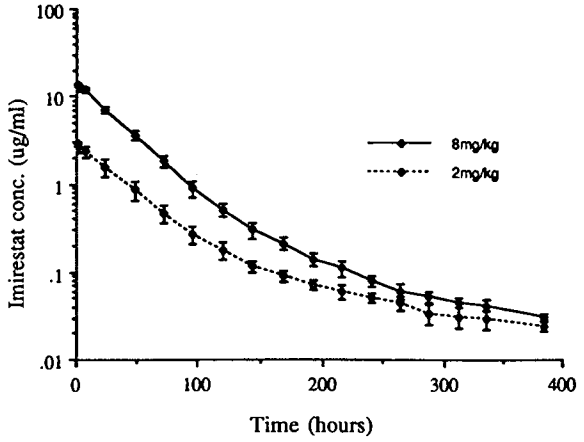

Fig. 2. Time course of imirestat plasma concentration in rats. Data are mean ± SD, $n = 6$. Imirestat was administered as a single bolus dose of either 2 or 8 mg/kg.

specific activity of 37.5 mCi/mmol (0.131 mCi/mg), and was >98% radiochemically pure by HPLC.

Animals and Treatments

Virus antibody-free male Sprague–Dawley rats of 250–300 g were obtained from Charles River Breeding Laboratories (Portage, MI) and were acclimatized for 2 days before undergoing any procedure. The animals had free access to food (Animal Specialities, Hubbard, OR) and water and were housed on corncob bedding in a temperature-controlled (22 ± 2°C) facility with a 12-hr light/dark cycle. For serial blood sampling and imirestat administration, each rat received a silastic cannula in the jugular vein, which was connected to PE-50 tubing and exteriorized at the back of the neck. For infusion of drugs, a PE-50/PE-100 cannula was placed just under the peritoneum, tunneled under the skin, and exteriorized at the back of the neck. All surgery was performed under xylazine/ketamine anesthesia. Rats were allowed 1–2 days to recover from anesthesia before use.

The dose dependence of imirestat pharmacokinetics was examined in two groups of nine rats which received either 2 or 8 mg/kg imirestat as a single iv bolus in a pH 9 (adjusted with dilute HCl) ethanol/propylene glycol/1 *M* NaOH (1/4/5) vehicle. The concentrations of the two doses were 1 and 4 mg/ml, and specific activities were 25.7 and 26.1 μCi/mg, respectively. Blood samples (0.2 ml) were collected 1, 4, 8, and 24 hr after the dose and every 24 hr thereafter for the next 14 days. The samples were centrifuged immediately and plasma was stored at −20°C until analysis. Earlier studies demonstrated that no metabolites were detectable in plasma by HPLC (10). Hence, the imirestat concentration in plasma was equal to the total radioactivity determined by scintillation counting.

The effect of statil or AL3152 administration on the pharmacokinetics of imirestat was first examined in three groups of six rats. Rats in each group received 0.2 mg/kg imirestat (0.1 mg/ml, 52.4 μCi/mg in pH 8, 10 m*M* L-arginine buffer). In addition, respective groups received ip bolus and ip infusions of vehicle (pH 8, 10 m*M* L-arginine), statil (2.4-mg/kg bolus and 56-μg/hr/kg infusion), or AL3152 (2-mg/kg bolus and 115-μg/hr/kg infusion). Based on published or preliminary pharmacokinetic data, these bolus and infusion regimens were expected to yield steady-state concentrations of approximately 4 μg/ml (12,13). In each case, the bolus was administered and the infusion begun 16 hr before imirestat administration. Infusions were continued throughout the period of blood sampling. Serial blood samples (~0.4 ml) were collected 1 hr after imirestat administration and every 24 hr for 15 days. Plasma was separated, stored, and analyzed for [^{14}C]imirestat as above.

To examine the effect of imirestat dose on the interaction with other aldose reductase inhibitors, the vehicle and AL3152 arms of the study above were repeated at a 2.0-mg/kg dose of imirestat iv. All other conditions, including blood sampling, were as described.

This treatment protocol was also used to determine the effect of the aldose reductase inhibitors statil and AL3152 on the tissue/plasma ratio of imirestat. Bolus doses and infusions of vehicle, statil, or AL3152 were administered to rats in three groups of six, and imirestat (0.2 mg/kg iv) was administered to each rat 16 hr after the bolus of competitor. Four rats in each treatment group were decapitated 24 hr after imirestat, and the remainder at 168 hr. Blood was collected from the neck; the eyes, adrenals, kidneys, lungs, brain, spleen, liver, testes, and heart were removed. The tissues were rinsed with ice-cold deionized water, blotted dry with lab tissue, and stored at −80°C until analyzed.

For analysis of [^{14}C]imirestat, organs were thawed at room temperature, weighed, and homogenized in 4 vol of ethanol/water (1/9) with a Polytron tissue homogenizer. Aliquots (1.0 ml) of homogenates of the eyes and adrenals were solubilized with 2.0 ml of Soluene-350, allowed to stand for 1 day at room temperature, and pH was adjusted to 7.0 to minimize chemiluminescence. Aliquots (0.1 ml) of plasma, tissue homogenates, or solubilized homogenates were added to 4 ml of scintillation cocktail (Readysafe, Beckman). Ra-

Table I. Pharmacokinetic Parameter Values of iv Imirestat in Rats[a]

Dose (mg/kg)	AUC (μg · hr/ml)	AUMC (mg · hr^2/ml)	MBRT (hr)	V_{ss} (L)	CL (ml/hr)
2	137 ± 12.7*	10.2 ± 1.64*	74.8 ± 12.5*	1.10 ± 0.228*	14.7 ± 1.38
8	531 ± 31.8	26.1 ± 3.79	49.2 ± 6.49	0.744 ± 0.103	15.1 ± 0.871

[a] Data are mean ± SD; $n = 6$. Imirestat was administered as either a 2- or an 8-mg/kg iv bolus.
* The parameter value is statistically different from the 8-mg/kg value, $P < 0.05$, Student's t test.

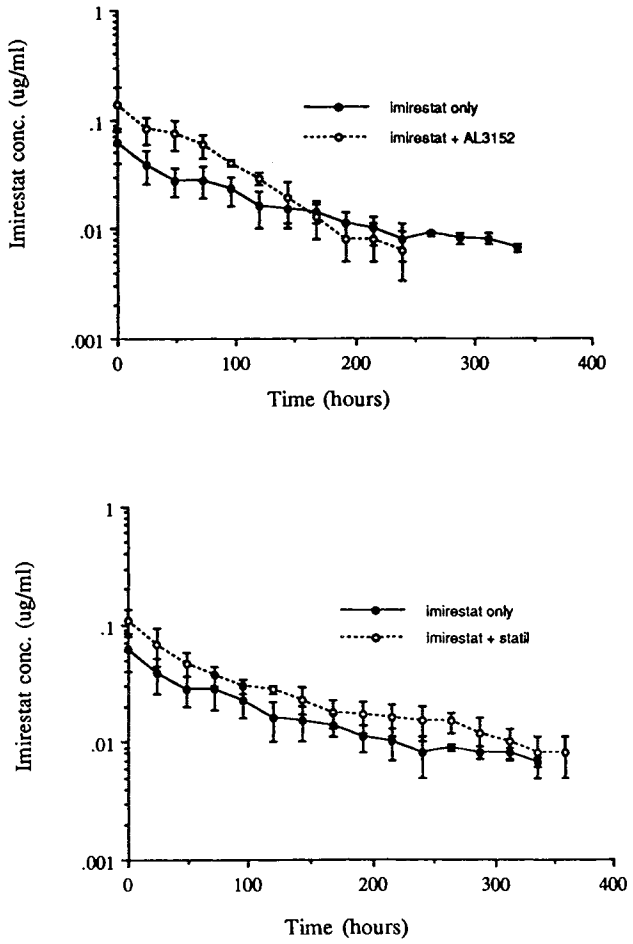

Fig. 3. Effect of AL3152 (top) and statil (bottom) on the plasma concentration–time course of imirestat (0.2 mg/kg, iv). Data are mean ± SD, $n = 6$. Drug dosing is described in the footnotes to Table II.

dioactivity was determined in a Packard Model 2000 Tri-Carb liquid scintillation analyzer when chemiluminescence had declined. Counting efficiency was determined by external standardization, and counting rates less than three times background were not further analyzed.

Data Analysis

Slopes of log plasma concentration–time curves were obtained by linear regression. Areas under the plasma concentration–time curves (AUC) and the first moment curve (AUMC), from time 0 to infinity, were estimated by linear trapezoidal summation, with extrapolation based on terminal half-life beyond the last data point. Clearance (Cl) was calculated as the ratio of dose/AUC, the mean body residence time (MBRT) as the ratio of AUMC/AUC, and the steady-state volume of distribution as the product of MBRT and Cl. Statistical comparisons were made with Student's t test with $\alpha = 0.05$.

RESULTS

The effect of dose on the time course of imirestat concentration in plasma is shown in Fig. 2. One hour after the dose of 8 mg/kg, the plasma concentration was 13.5 ± 0.74 μg/ml (mean ± SD), and at the same time after the 2-mg/kg dose, it was 2.87 ± 0.21 μg/ml. Thus, at this time the ratio of concentration for the two doses was 4.7, slightly greater than the fourfold ratio expected if drug distribution were independent of dose. The early plasma level–time courses of the two doses do not decline in parallel. At 264 hr, the ratio of plasma concentrations was 1.7. Interestingly, the slopes of the two curves were not significantly different ($P > 0.25$) after 264 hr, when the plasma concentration was <0.05 μg/ml (-0.00767 ± 0.0004 hr^{-1} at the high dose and -0.00755 ± 0.001 hr^{-1} at the low dose).

Pharmacokinetic parameter values for the two doses are listed in Table I. Clearance was not different between the two doses, while V_{ss} declined by 32% and MBRT decreased by 34%.

The effect of concomitant administration of a second aldose reductase inhibitor on the plasma concentration–time course of 0.2 mg/kg imirestat is shown in Fig. 3. The highest concentration of imirestat at this dose was approximately 0.06 μg/ml and declined slowly over the next 336 hr. When AL3152 was added, the highest concentration of imirestat observed was approximately doubled. Also, the slope of the decline of the log of imirestat plasma concentration became more steep, from -0.0075 ± 0.001 hr^{-1} in the absence of AL3152 to -0.013 ± 0.004 hr^{-1} when AL3152 was added (P

Table II. Effect of AL3152 and Statil on the Pharmacokinetic Parameter Values of 0.2-mg/kg Imirestat in Rats[a]

Competitor	AUC (μg · hr/ml)	AUMC (mg · hr²/ml)	MBRT (hr)	V_{ss} (L)	CL (ml/hr)
None[b]	6.51 ± 2.08	0.924 ± 0.319	138 ± 30.5	4.49 ± 1.12	33.5 ± 10.7
AL3152[c]	10.5 ± 2.10*	0.896 ± 0.349	84.6 ± 22.0*	1.66 ± 0.448*	19.7 ± 3.74*
Statil[d]	10.5 ± 1.89*	1.50 ± 0.436*	142 ± 29.8	2.75 ± 0.632*	19.6 ± 4.11*

[a] Data are mean ± SD; $n = 6$.

[b] Imirestat was administered as a single 0.2-mg/kg iv bolus 16 hr after an ip infusion of 1 mM, pH 8.0, arginine-buffered solution (0.2 ml/hr) was begun and maintained throughout the study.

[c] AL3152 was administered as a 2-mg/kg ip bolus 16 hr before imirestat (0.2 mg/kg, iv). An ip infusion of AL3152 (115 μg/kg · hr) was begun with the injection and maintained throughout the study.

[d] Statil was administered as a 2.4-mg/kg ip bolus 16 hr before imirestat. An ip infusion of statil (56 μg/hr · kg) was begun with the injection and maintained throughout the study.

* The mean of the parameter value is statistically different from the control value, $P < 0.05$, Student's t test.

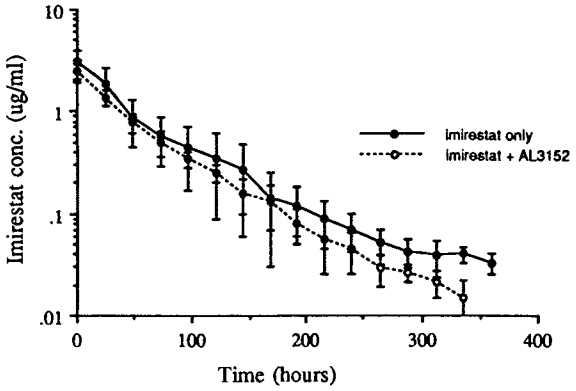

Fig. 4. Effect of AL3152 on the plasma concentration–time course of imirestat (2.0 mg/kg, iv). The data are mean ± SD, $n = 6$. AL3152 dosing is described in Table II, footnote *c*.

< 0.005). Somewhat in contrast, statil administration increased imirestat plasma concentration but did not alter the slope of the plasma concentration–time profile.

The summary of the effect of other aldose reductase inhibitors on the pharmacokinetics of 0.2-mg/kg imirestat is given in Table II. Both drugs decreased the Cl of imirestat by approximately 40%. AL3152 decreased the V_{ss} of imirestat by 63%, while statil decreased the imirestat V_{ss} by 39%. The opposing influences of Cl and V_{ss} on MBRT resulted in a 39% decline in imirestat MBRT in the presence of AL3152, while statil did not significantly alter imirestat MBRT. It is also clear that, when the dose of imirestat is reduced to one-tenth the lower dose in Table I, the volume of distribution increases approximately fourfold and clearance twofold (Table II).

The effect of AL3152 on the plasma concentration–time profile and pharmacokinetics of imirestat was abolished when the imirestat dose was raised to 2.0 mg/kg (Fig. 4 and Table III).

Table IV shows the effect of AL3152 and statil on the tissue/plasma ratio of imirestat 24 hr and 168 hr after 0.2-mg/kg imirestat. In the control (no-competitor) and treatment groups, the highest tissue/plasma ratios were found in the adrenals, testes, kidneys, heart, and eyes. AL3152 decreased the imirestat tissue/plasma ratio by 50–75%, while statil decreased it by 25–50%.

DISCUSSION

Pharmacokinetic theory holds that clearance is independent of tissue binding and that V_{ss} will decrease with increasing free fraction of drug in tissue. It was expected therefore that MBRT would decrease with increasing tissue free fraction. On the basis of this theoretical framework, the decrease in V_{ss} and MBRT with increasing dose of imirestat and the dose independence of clearance (Table I) are consistent with a saturable tissue binding phenomenon. Similarly, the convergence of the plasma concentration–time curves following 2- and 8-mg/kg imirestat doses are also consistent with the previously reported persistence of imirestat in tissue (10). As the dose is decreased to 0.2 mg/kg (Table II), V_{ss} increases fourfold and clearance twofold.

The other studies reported here also support the hypothesis that imirestat pharmacokinetics are governed in part by a saturable tissue binding phenomenon. Statil and AL3152 both decreased the V_{ss} of 0.2-mg/kg imirestat. The effect of these drugs on clearance is apparently mediated through another mechanism, e.g., inhibition of imirestat metabolism (<1% of dose is eliminated intact) (8). As would be expected for a competitive binding process, the effect of AL3152 on V_{ss} was abolished when the imirestat dose was increased 10-fold (Table III). At the higher dose of imirestat, AL3152 administration did not alter imirestat clearance, suggesting that this effect was also at least partly competitive. Direct analysis of tissue/plasma concentration ratio (Table IV) confirmed the displacement of imirestat from tissues.

No direct information on the tissue-binding site of imirestat was obtained in this study, but competitive displacement by other aldose reductase inhibitors (one structurally similar, the other not) suggests that the binding may be to the target enzyme. If this is the case, the duration of action may be substantially longer than would be expected by the initial rate of decline of imirestat plasma concentration. A similar phenomenon has been observed with omeprazole, which is a very potent inhibitor of an ATPase specific to a K^+/H^+ antiporter in the stomach (15). Autoradiography demonstrates the persistence of omeprazole in the stomach well after the disappearance of the drug from plasma (14). The duration of effect of omeprazole is consistent with its persistence in the stomach wall (16,17).

The spirohydantoin aldose reductase inhibitors are extremely potent, with median inhibitory concentrations of the order of 10^{-9}–10^{-8} *M* (6,7,11). As with many new drugs, they have been selected as therapeutic prospects because of their high potency. Since *in vitro* potency is often an important criterion for selection of therapeutic candidates, the tissue binding pharmacokinetic nonlinearity reported here for imirestat may become a commonplace observation.

Table III. Effect of AL3152 on the Pharmacokinetic Parameter Values of 2.0-mg/kg Imirestat in Rats[a]

Competitor	AUC (μg · hr/ml)	AUMC (mg · hr²/L)	MBRT (hr)	V_{ss} (L)	CL (ml/hr)
None[b]	160 ± 72.0	9.50 ± 4.56	59.7 ± 9.5	0.888 ± 0.462	14.7 ± 5.87
AL3152[c]	122 ± 23.1	6.64 ± 1.52	54.5 ± 6.00	0.925 ± 0.185	17.0 ± 3.57

[a] Data are mean ± SD; $n = 6$. All parameter values are not statistically different, $P > 0.05$, Student's *t* test.
[b] Imirestat was administered as a single 2-mg/kg iv bolus. An infusion for the control group was administered as in Table II.
[c] AL3152 was administered as in Table II.

Table IV. Effect of AL3152 and Statil on the Tissue-to-Plasma Imirestat Concentration Ratios[a]

	Competitor[c]					
	None		AL3152		Statil	
Tissue[b]	24 hr	168 hr	24 hr	168 hr	24 hr	168 hr
Eye	11.2 ± 3.48	11.3 ± 1.39	3.22 ± 0.746	5.65 ± 0.537	7.82 ± 2.78	6.81 ± 2.65
Adrenal	56.9 ± 20.0	49.1 ± 15.1	12.3 ± 2.59	19.3 ± 2.81	17.7 ± 1.27	22.0 ± 6.49
Kidney	18.0 ± 2.17	19.6 ± 2.91	3.28 ± 0.934	3.15 ± 0.841	12.3 ± 3.23	11.5 ± 4.56
Testes	21.2 ± 1.60	29.2 ± 2.87	2.39 ± 0.450	3.34 ± 0.451	14.1 ± 2.07	20.2 ± 5.35
Lung	7.47 ± 1.67	8.98 ± 0.656	1.90 ± 0.227	—[d]	4.39 ± 1.22	5.71 ± 0.960
Brain	3.37 ± 0.585	2.87 ± 0.441	1.03 ± 0.159	—[d]	1.95 ± 0.249	2.10 ± 0.289
Spleen	8.99 ± 1.56	9.88 ± 2.03	2.34 ± 0.208	4.15 ± 1.22	5.42 ± 1.37	5.85 ± 1.38
Liver	7.77 ± 0.981	8.03 ± 1.53	2.94 ± 0.261	3.99 ± 1.08	6.27 ± 1.01	5.45 ± 1.42
Heart	13.7 ± 1.26	16.1 ± 4.09	2.55 ± 0.415	4.03 ± 0.470	7.36 ± 1.38	10.9 ± 3.27

[a] Data are mean ± SD; $n = 5$ for the control, $n = 4$ for the statil group, and $n = 3$ for the AL3152 group.
[b] The plasma concentrations fo imirestat at each corresponding time point for the control, statil, and AL3152 groups are as follows: at 24 hr, 0.034 ± 0.004, 0.05 ± 0.008, and 0.097 ± 0.011 μg/ml, respectively; at 168 hr, 0.015 ± 0.001, 0.018 ± 0.003, and 0.011 ± 0.001 μg/ml, respectively.
[c] Drugs were administered as described in Table II.
[d] Below detection limits.

ACKNOWLEDGMENT

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