

Relative Potencies and Additivity of Perchlorate, Thiocyanate, Nitrate and Iodide on the Inhibition of Radioactive Iodide Uptake by the Human Sodium Iodide Symporter

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The presence of perchlorate (ClO_4^-) in some U.S. drinking water supplies has raised concern about potential adverse thyroidal health effects, because ClO_4^- is known to competitively inhibit iodide uptake at the sodium-iodide symporter (NIS). Humans are nutritionally and environmentally exposed to other competitive inhibitors of iodide uptake, including thiocyanate (SCN^-) and nitrate (NO_3^-). The joint inhibiting effects of these three anions was studied by exposing Chinese hamster ovary cells stably expressing human NIS to varying concentrations of each anion separately, and in combination, and conducting measurements of ^{125}I uptake. The entire data set was fit to a single Hill equation using maximum likelihood. The relative potency of ClO_4^- to inhibit ^{125}I uptake at the NIS was found to be 15, 30 and 240 times that of SCN^- , I^- and NO_3^- respectively on a molar concentration basis, with no evidence of synergism. These results are consistent with a common mode of action by these anions of simple competitive interaction, in which a concentration of any one of ClO_4^- , SCN^- , and NO_3^- , occurring either individually or as part of a mixture of the three anions, is indistinguishable from a concentration or dilution of either one of the remaining two ions in inhibiting iodine uptake at the sodium-iodide symporter

Key Words: sodium iodide symporter, cell culture, perchlorate, thiocyanate, iodine

INTRODUCTION

A number of different inorganic anions can block iodide (I^-) uptake at the thyroidal sodium-iodide symporter (NIS) in a competitive manner. Of these, nitrate (NO_3^-), thiocyanate (SCN^-), and perchlorate (ClO_4^-) are of particular dietary and/or environmental importance. Sufficient inhibition of iodide uptake can lead to decreased thyroid hormone production and, ultimately, result in adverse health effects secondary to hypothyroxinemia.

NO_3^- is ubiquitous in foods, either occurring naturally, as in green leafy vegetables, or added as a preservative, as in processed meats. NO_3^- is also very common in both surface and ground sources of drinking water, primarily due to the agricultural use of nitrate fertilizers. The average daily intake of NO_3^- is estimated to be about 40 – 50 mg/day for adults, based on studies in Denmark and the United Kingdom (1-3). Typical serum levels in the western world range from 10 – 140 $\mu\text{mol/l}$ with a mean of 30 – 50 $\mu\text{mol/l}$ (3-9), and the serum half life is 5 – 8 hours (10-12).

Foods that are particularly rich in thiocyanate include cabbage, broccoli, Brussels sprouts, turnips, rapeseed and mustard seed (13-16). Cauliflower, cabbage, radishes, spinach and tomatoes have been reported to contain on average 88, 86, 7, 5, and 2 mg/kg

(wet weight) respectively of SCN^- (17). Milk contains 2 – 10 mg/l SCN^- derived from dairy cattle grazing on plants from the cruciferous family (17-22). In some areas of the world, SCN^- is added to milk as a preservative (23). Cassava, which can contain the equivalent of as much as 3,400 mg/kg SCN^- on a dry weight basis, is the most important dietary source of SCN^- in some areas of the world (24). In areas that are not dependent upon cassava as a staple food, cyanide from cigarette smoking is the most important source of SCN^- in the body. Non-smokers' serum levels are typically 10 – 70 $\mu\text{mol/l}$, compared to a typical range of 80 – 120 $\mu\text{mol/l}$ among smokers (13, 25-31). SCN^- has a reported "kidney threshold" at a serum level of 200 – 300 $\mu\text{mol/l}$ (24). Serum half-life at levels over 200 – 300 $\mu\text{mol/l}$ has been determined to be about 3 days among healthy volunteers (32), while serum half-life among cigarette smokers who quit (initial SCN^- concentrations of 130 – 186 $\mu\text{mol/l}$) has been estimated to be about 6 days (33).

Drinking water supplies in some parts of northern Chile contain naturally-occurring ClO_4^- at average levels ranging up to more than 100 $\mu\text{g/l}$ (34). Since 1997, ClO_4^- has been detected in U.S. underground and surface water supplies, primarily in the southwest, in concentrations generally below 50 $\mu\text{g/l}$ (35). These

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levels are thought to be mainly due to military and industrial production and use of ammonium perchlorate. ClO_4^- is a natural contaminant in NO_3^- fertilizers mined in Chile that are widely used in the U.S. in tobacco and organic farming. The half-life of ClO_4^- in human serum has been estimated to be about 8 hours (36).

The relative effects of ClO_4^- , SCN^- and NO_3^- on radioactive iodide uptake (RAIU) inhibition and upon RAI discharge were evaluated in rats. It was shown that, on a serum molar concentration basis, ClO_4^- was approximately 10 times as potent as SCN^- and about 300 times as potent as NO_3^- in inhibiting RAIU in the thyroid and that SCN^- was slightly more potent than I^- . All three treatments resulted in increased thyroid weight, decreased thyroid iodide and follicular hyperplasia (37, 38). In another study in rats, the relative molar doses of ClO_4^- , SCN^- , and NO_3^- for causing 50% increase in thyroid weight were 1:20:550. The molar concentrations of ClO_4^- , SCN^- and NO_3^- required to produce a 50% inhibition in the concentration of radio-labeled iodide in thyroid slices in vitro were determined to be in a ratio of 1:20:400 (39). The concentrations of ClO_4^- , SCN^- , and NO_3^- required to decrease the uptake of radio-labeled iodide in isolated rat thyroids by 50% was determined to be in a molar ratio of 1:20:240 (40).

Two separate studies with Chinese Hamster Ovary (CHO) cell lines stably transfected with human NIS demonstrated that ClO_4^- and SCN^- inhibited RAIU in a dose-dependent manner, reaching nearly complete inhibition at 20 $\mu\text{mol/l}$ and 500 $\mu\text{mol/l}$, respectively. ClO_4^- was nearly 10 times as potent as SCN^- in inhibiting RAIU throughout the range of concentrations tested (41). In these two studies, 5 to 10-fold dilutions of normal serum was demonstrated to inhibit RAIU up to 20 – 25% (41, 42).

Thus in vivo and in vitro studies are in substantial agreement, indicating that, on a molar concentration basis, ClO_4^- is 10 to 20 times more potent than SCN^- and several hundred times more potent than NO_3^- in inhibiting RAIU, with unlabeled iodide having roughly the same potency as SCN^- . However the combined effect of the simultaneous administration of these inhibitors has not been investigated in any detail in these studies.

In the present study, we use data from an in vitro test system in which human NIS was stably transfected into a CHO cell line to investigate the simultaneous joint effects of ClO_4^- , SCN^- , NO_3^- , and (non-radioactive) I^- in inhibiting RAIU. In particular, we investigate 1) whether the concentration-RAIU curves for these anions are the same or different (after adjusting for differences

in relative potency); and 2) whether there is evidence for non-additive interactions (synergistic or antagonistic) among these RAIU inhibitors.

Table 1 - Design of 26 Experiments Measuring RAIU in CHO Cells Transfected with Human NIS

Exp. No.	Concentrations ($\mu\text{mol/l}$)			
	SCN	NaI	NO_3^-	ClO_4^-
1				CUR10: 0.1 - 100
2	CUR10: 0.1 - 100			
3			CUR7: 0.1 - 10000	
4				CUR9: 0.1 - 12.8
5	CUR9: 1 - 128			
6			CUR9: 10 - 1280	
7		1		CUR9: 0.1 - 12.8
8	CUR9: 1 - 128	1		
9		1	CUR9: 10 - 1280	
10		10		CUR9: 0.1 - 12.8
11	CUR9: 1 - 128	10		
12		10	CUR9: 10 - 1280	
13A				CUR9: 0.1 - 12.8
13B			10	CUR9: 0.1 - 12.8
14A				CUR9: 0.1 - 12.8
14B			40	CUR9: 0.1 - 12.8
15A				CUR9: 0.1 - 12.8
15B			160	CUR9: 0.1 - 12.8
16A	CUR9: 1 - 128			
16B	CUR9: 1 - 128		10	
17A	CUR9: 1 - 128			
17B	CUR9: 1 - 128		40	
18A	CUR9: 1 - 128			
18B	CUR9: 1 - 128		160	
19A		10		CUR9: 0.1 - 12.8
19B		10	10	CUR9: 0.1 - 12.8
20A		10		CUR9: 0.1 - 12.8
20B		10	40	CUR9: 0.1 - 12.8
21A		10		CUR9: 0.1 - 12.8
21B		10	160	CUR9: 0.1 - 12.8
22A	CUR9: 1 - 128	10		
22B	CUR9: 1 - 128	10	10	
23A	CUR9: 1 - 128	10		
23B	CUR9: 1 - 128	10	40	
24A	CUR9: 1 - 128	10		
24B	CUR9: 1 - 128	10	160	
25A		1		CUR9: 0.1 - 12.8
25B	2	1		CUR9: 0.1 - 12.8
25C	8	1		CUR9: 0.1 - 12.8*
25D	18.5	1		CUR9: 0.1 - 12.8*
26A	2	1		CUR9: 0.1 - 12.8
26B	8	1		CUR9: 0.1 - 12.8
26C	18.5	1		CUR9: 0.1 - 12.8

Key: E.g., CUR9: 0.1 - 12.8 signifies a concentration-uptake response investigation involving 9 distinct concentrations, including zero, with the 8 positive concentrations being distributed between 0.1 and 12.8 $\mu\text{mol/l}$.

* Group exposed to zero concentration of ClO_4^- was not exposed to SCN.

METHODS

Experimental

The experimental protocol was very similar to that used previously as part of a study to investigate effects of auto-antibodies on the NIS (41) and performed

in the same laboratory. One hundred thousand cells from a CHO cell line into which human NIS had been stably transfected were seeded in 24-well plates and cultured in Dulbecco Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS). When cells reached 100% confluence, the medium was removed, and cells were washed with physiologic solution and combined with 500 ml buffer A (Hanks' balanced salt solution (HBSS) containing 0.5% BSA (bovine serum albumin) and 10 mmol HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid), pH 7.4) containing 100,000 counts per minute (cpm) of carrier-free Na¹²⁵I (0.1 mCi, 0.4 nmol/l). This mixture was separated into wells, and various concentrations of one of three RAIU inhibitors (SCN⁻, NO₃⁻, or ClO₄⁻) were added to individual wells to provide information on concentration-uptake response for that anion. Each experimental concentration was duplicated in two separate wells. In a number of cases, fixed molar concentrations of other RAIU inhibitors (including non-radioactive iodide) were added prior to separating the mixture into wells. After incubation at 37°C for 45 minutes, cells were quickly washed twice with 2 ml of ice-cold buffer A, and then solubilized with 1 ml of 0.1 mol/l sodium hydroxide. ¹²⁵I uptake was determined by measuring the radioactivity cpm from each well using a γ -counter.

Data collected at the same time starting with the same basic mixture of incubated cells and ¹²⁵I are identified as comprising the data from a single "experiment". The designs of separate experiments included in the present study are shown in Table 1. Except for the three range finding experiments (Experiments 1 – 3), concentrations in each concentration-uptake response consisted of consecutive doubles of the lowest positive concentration.

Data analysis

A common method of evaluating data from RAIU studies is to plot the relative radioactive uptake (uptake at a given dose divided by the uptake at zero concentration) and to use this plot to estimate the concentration that produces 50% inhibition of iodide uptake. However, a somewhat more detailed approach is required here. First, the goals of the present analysis include determining whether the different anions inhibit uptake via a common concentration response (once differences in potencies are accounted for) and whether the presence of one anion (e.g., SCN⁻) potentiates or inhibits the effect of another anion (e.g., ClO₄⁻). This requires comparing the shapes of uptake response curves

in addition to estimating 50% uptake inhibition concentrations. Second, not all of the experiments included a true background point (i.e., a well in which none of the four RAIU inhibitors were added), in which case there is no denominator readily available for direct computation of percent RAIU. All of these issues can be addressed by application of a statistical model of uptake inhibition. The statistical model used in the present analysis is described below.

A form of the Hill model, which is often used to model drug action (43), is used to model the competitive inhibition of thyroidal iodide uptake. Let X_T, X_N, X_P, X_I, (where T = SCN⁻, N = NO₃⁻, P = ClO₄⁻, and I = non-radioactive I⁻) denote the concentrations (μ mol/l) of the respective anions applied to a single well in an experiment, and let C be the corresponding cpm recorded on the γ -counter. The expected cpm, C_{expected}, is assumed to be related to the anion concentrations according to the following model:

$$C_{\text{expected}} = \frac{C_0}{1 + \left(\frac{X_T}{T_{50}} + \frac{X_I}{I_{50}} + \frac{X_N}{N_{50}} + \frac{X_P}{P_{50}} \right)^r} \quad (\text{eq. 1A})$$

where T₅₀, I₅₀, N₅₀, P₅₀ are the concentrations of SCN⁻, I⁻, NO₃⁻, and ClO₄⁻, respectively, that, when applied alone, result in 50% inhibition of RAIU. To see this, note that if, e.g., SCN⁻ is present at the level, X_T = T₅₀, and no other anion is present (X_N = X_P = X_I = 0), then eq. 1 becomes

$$C_{\text{expected}} = \frac{C_0}{1 + \left(\frac{T_{50}}{T_{50}} + \frac{0}{I_{50}} + \frac{0}{N_{50}} + \frac{0}{P_{50}} \right)^r} = \frac{C_0}{1 + (1)^r} = 0.5C_0$$

The parameter C₀ represents the expected background count in the absence of any RAIU inhibitor, and r is a shape parameter that influences the curvature of the concentration response curve. In the implementation of the model, C₀ is fixed for a given experiment but allowed to vary from experiment to experiment to reflect differences in experimental conditions, which might include such factors as changes over time in radioactivity of ¹²⁵I, different initial numbers of cells, and differences in environmental conditions such as temperature, etc. All other parameters are assumed to be the same for all experiments.

The model (eq. 1A) can be written in the equivalent form

$$C_{\text{expected}} = \frac{C_0 \times P_{50}^r}{P_{50}^r + (\beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P)^r} \quad (\text{eq. 1B})$$

where the β 's represent the relative potencies of the respective anions (relative to ClO_4^-) for inhibiting iodide uptake, i.e., $\beta_T = P_{50}/T_{50}$, $\beta_N = P_{50}/N_{50}$, and $\beta_I = P_{50}/I_{50}$. The expected relative uptake can be written as

$$\begin{aligned} RU_{\text{expected}} &= \frac{C_{\text{expected}}}{C_0} \\ &= \frac{P_{50}^r}{P_{50}^r + (\beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P)^r} \quad (\text{eq. 2}) \\ &= \frac{P_{50}^r}{(P_{50}^r + \text{PEC}^r)} \end{aligned}$$

where the PEC (perchlorate equivalent concentration) is given by

$$\text{PEC} = \beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P \quad (\text{eq. 3})$$

This model assumes that each of the four anions has exactly the same effect as a dilution or concentrate of any of the other three anions. E.g., the effect of X_T $\mu\text{mol/l}$ of SCN^- is exactly the same as $\beta_T \times X_P$ $\mu\text{mol/l}$ of ClO_4^- . The PEC of a mixture is the equivalent concentration of ClO_4^- that would cause the same inhibition of iodide uptake as the mixture.

In order to test for possible interaction (e.g., synergy or antagonism) between two anions in inhibiting RAIU, the model was augmented by adding a term to the PEC equal to the product of the molar concentrations of the two anions times an interaction parameter, β_{INT} . If β_{INT} is different from zero, the model no longer predicts that an RAIU inhibitor acts exactly as a concentrate or dilution of another inhibitor. Positive values of β_{INT} indicate a synergistic interaction in which the presence of one inhibitor potentiates the inhibitory effect of the other inhibitor, whereas negative values of β_{INT} indicate an antagonistic (opposite to synergistic) effect.

The model was fit by maximum likelihood to the data from each of the experiments using the statistical model, $C = C_{\text{expected}} + \varepsilon$, where ε for different wells are independent and normally distributed, with a constant standard deviation, σ . Likelihood ratio tests were used to test hypotheses and statistical confidence limits were computed by the profile likelihood method (44, 45). Graphical representations of the fit of the model to the relative uptake data were developed by plotting the raw counts divided by the model-derived expected count in

the absence of inhibitory anions (C/C_0), and comparing these ratios to curves of the model prediction of relative uptake (eq. 2).

RESULTS

Allowing the background cpm (C_0) to vary with experiment produced a highly significant improvement in fit over assuming a fixed background applicable to all experiments (chi-square = 1639, 25 df, $p < 0.00001$), which indicates, as expected, the presence of significant heterogeneity in absolute cpm from different experiments. Table 2 indicates that fitting the model with the shape parameter, r , estimated (best estimate $r = 1.04$) or with r fixed at $r = 1$ produce very similar results. These two model fits produce very similar estimates of the 50% RAIU inhibition concentrations, and the variations in the data around the two model fits are almost identical ($\sigma = 1716$ cpm with $r = 1.04$ and that the difference in RAIU predicted by the two models is never larger than 1% for any PEC. Thus, although allowing r to be different from 1.0 provides a statistically significant improvement in the fit ($p = 0.02$), this improvement is inconsequential biologically. Consequently, we henceforth consider only the reduced model with r fixed at $r = 1.0$. According to this model, on a molar basis, ClO_4^- was 15 ($1/\beta_T$), 30 ($1/\beta_I$) and 240 ($1/\beta_N$) times more potent than SCN^- , I^- , and NO_3^- , respectively, in inhibiting RAIU.

Table 2 - Results from Applying Model for RAIU Inhibition (eq. 1) to Data from All Experiments

	r estimated		r = 1	
	90% C.I.		90% C.I.	
Concentrations ($\mu\text{mol/l}$) corresponding to 50% inhibition				
P_{50}	1.27	(1.23, 1.32)	1.22	(1.19, 1.26)
T_{50}	19.3	(18.4, 20)	18.7	(17.9, 19.3)
I_{50}	33.9	(29.8, 35.8)	36.6	(29.3, 38.6)
N_{50}	297	(283, 312)	293	(279, 309)
Potencies relative to ClO_4^-				
β_P	1 ^a		1 ^a	
$\beta_T (= P_{50}/T_{50})$	0.0660	(0.063, 0.0692)	0.0656	(0.0625, 0.0688)
$\beta_I (= P_{50}/I_{50})$	0.0376	(0.035, 0.0404)	0.0335	(0.0313, 0.0358)
$\beta_N (= P_{50}/N_{50})$	0.00429	(0.00403, 0.00455)	0.00417	(0.00392, 0.00444)
r	1.04	(1.02, 1.08)	1	
σ (cpm)	1716		1722	

Code: P - ClO_4^- ; T - SCN^- ; I - NaI ; N - NO_3^- .

^a By definition, since potencies are computed relative to ClO_4^- .

Figure 1 shows the relative RAIU (observed cpm divided by the estimated mean background count, C_0) as a function of the molar concentration, using only

concentration response data involving a single inhibitor (Table 1: Experiments 1-6, and “A” portions of Experiments 13 – 18). This figure shows clearly that ClO_4^- was the most potent inhibitor on a molar basis, followed by SCN^- , and then NO_3^- . The three curves shown in the figure were based on the single model fit to the entire database reported in Table 2 ($r=1$). This single model appears to describe well the concentration-responses of the respective anions. The molar concentrations in Table 2 corresponding to 50% inhibition of iodide uptake can be visualized in this graph by dropping vertically from where a curve crosses the 50% uptake line to the (molar concentration) x-axis.

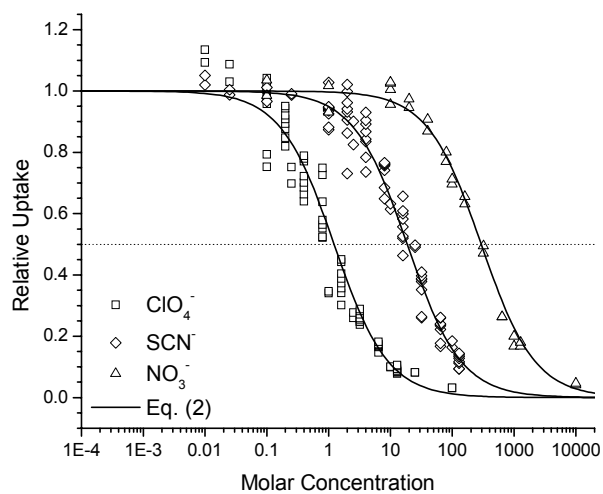


Figure 1 - Relative Uptake of SCN^- , ClO_4^- and NO_3^- as a Function of Molar Concentration

Figure 2 shows the same data as in Figure 1, but plotted as a function of the ClO_4^- equivalent concentration (PEC). Intuitively, Figure 2 is derived from Figure 1 by sliding the data for SCN^- and NO_3^- to the left until they match up with those for ClO_4^- . The amount (factor) by which data points for SCN^- must be shifted to match those for ClO_4^- is a measure of the relative potency (β_T) of SCN^- . Although the β 's could be estimated in this intuitive manner, they were in fact estimated by the more precise statistical method of maximum likelihood. Figure 2 confirms that the data, when plotted as a function of the PEC, are well-described by a single curve.

The data points involving concentrations of multiple RAIU inhibitors can be added to the graph in Figure 2 by computing the PEC for each concentration combination. Figure 3 shows the resulting graph when

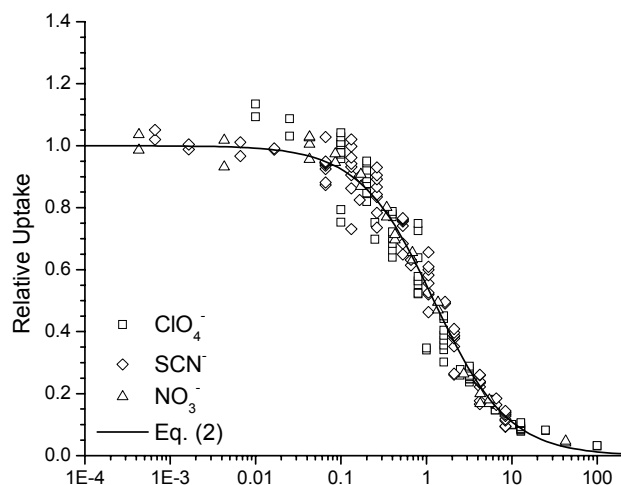


Figure 2 - Relative Iodine Uptake as a Function of Perchlorate Equivalent Concentration (PEC) for

all data points involving concentration of any inhibitor(s) are plotted as a function of the PEC. This figure demonstrates that the totality of the data can be adequately described by a single curve when concentrations are expressed in terms of the PEC.

Statistical tests for interactions (synergistic or antagonistic) between anion pairs [SCN^- , ClO_4^-], [NO_3^- , ClO_4^-], and [SCN^- , NO_3^-] indicated that in each case the estimate of the interaction parameter was slightly negative (indicative of antagonism rather than

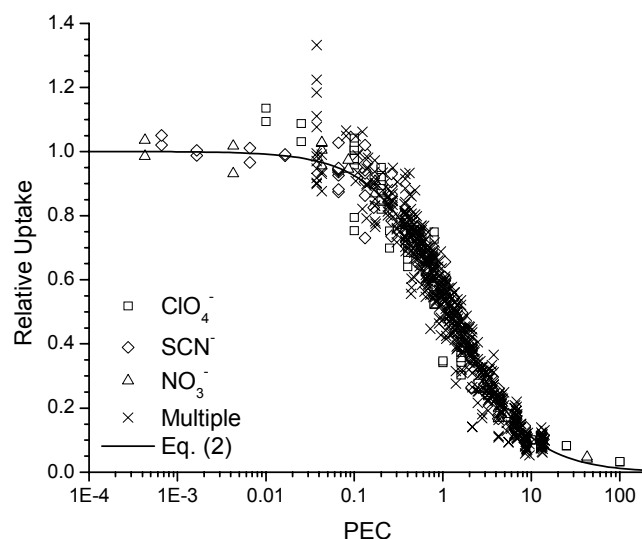


Figure 3 - Relative Iodine Uptake as a Function of Perchlorate Equivalent Concentration (PEC) for All Data

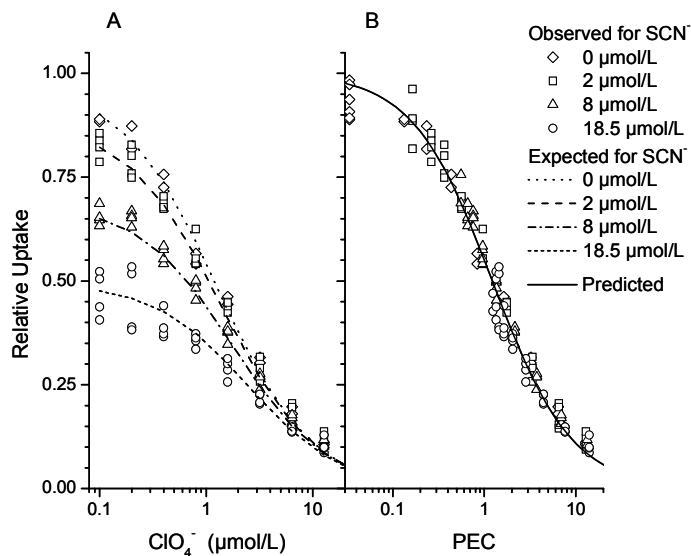


Figure 4 - Relative Iodine Uptake as a Function of the Molar Concentration of ClO_4^- (A) or the Perchlorate Equivalent Concentration (PEC) (B), for Four Background Concentrations of SCN^- (All in Presence of 1 $\mu\text{mol NaI}$)

synergism), but in no case significantly different from zero ($p \geq 0.18$ in all cases). Figures 4 - 6 show data from experiments that generated the concentration response for a single RAIU inhibitor in the presence of fixed concentrations of other RAIU inhibitors. Figure 4

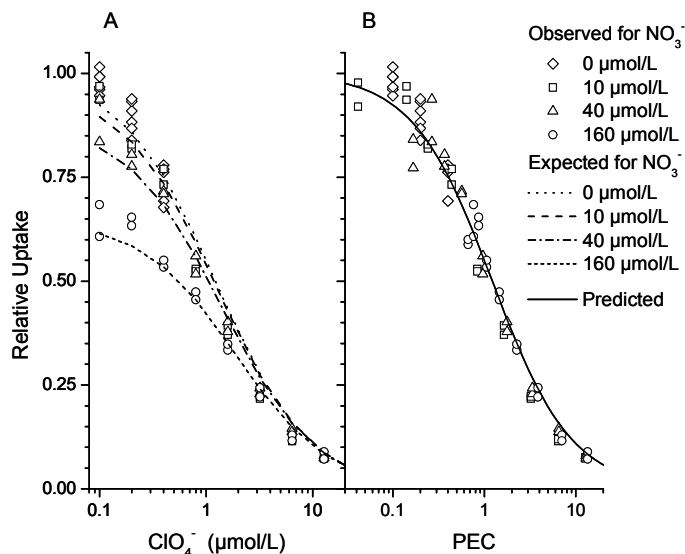


Figure 5 - Relative Iodine Uptake as a Function of the Molar Concentration of ClO_4^- (A) or the Perchlorate Equivalent Concentration (PEC) (B), for Four Background Concentrations of NO_3^-

is based on data from Experiments 25 and 26 (Table 1) in which concentration-response data for ClO_4^- were generated at four concentrations of SCN^- , (0, 2, 8, and 18.5 $\mu\text{mol/L}$), all in the presence of 1 $\mu\text{mol/L NaI}$. Figure 4A shows the experimental relative RAIU values plotted against the ClO_4^- molar concentration, whereas Figure 4B shows the same RAIU data plotted against the PEC. In both figures, the solid curves are the expected values for relative RAIU, based upon the fit of the model (eq. 1) derived from all 26 studies (Table 2, $r = 1$). Figure 5 shows similar plots of concentration-response data for ClO_4^- generated in the

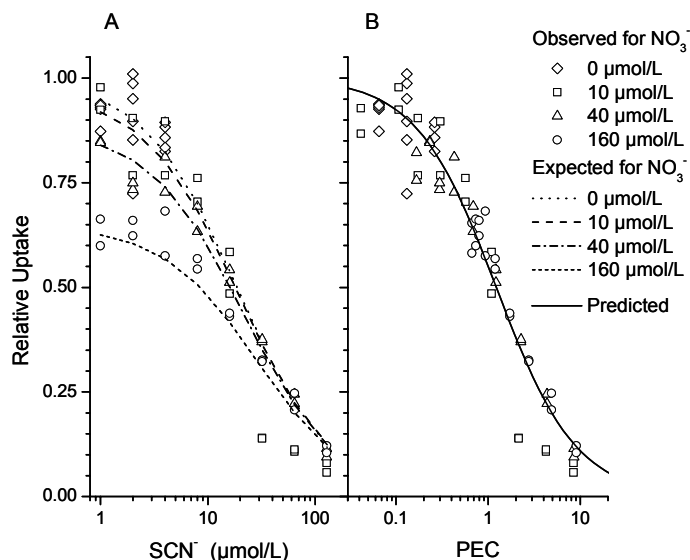


Figure 6 - Relative Iodine Uptake as a Function of the Molar Concentration of SCN^- (A) or the Perchlorate Equivalent Concentration (PEC) (B), for Four Background Concentrations of NO_3^-

presence of different concentrations of NO_3^- (Experiments 13, 14 and 15), and Figure 6 shows similar plots of concentration-response data for SCN^- generated in the presence of different concentrations of NO_3^- (Experiments 16, 17, 18). A synergistic effect between two anions would manifest itself in these graphs by a tendency for the relative RAIU concentration-response values to fall below the model predictions at the higher concentrations of other anion, and above the model predictions at the lower concentrations. No such tendency is apparent in any of these graphs, which is consistent with lack of evidence for synergism among ClO_4^- , SCN^- and NO_3^- from the statistical analysis.

DISCUSSION

The present study investigated the joint effects of simultaneous exposure to multiple RAIU inhibitors in some detail by developing numerous concentration-response curves for specific RAIU inhibitors (ClO_4^- , SCN^- , and NO_3^-) generated in the presence of fixed concentrations of other inhibitors (Table 1). These data were examined for non-additive interactions (either synergism or antagonism) using both formal statistical tests and graphical methods (Figures 4 – 6). Neither of these approaches found evidence of either synergism or antagonism. Rather, the data indicated that these RAIU inhibitors interact in a simple additive fashion. The concentration-response for each inhibitor was indistinguishable from that of each of the others, once adjusted for differences in inhibition potencies; i.e., the effect of each of the RAIU inhibitors was indistinguishable from either a concentrate or dilution of any one of the other inhibitors. Correspondingly, the inhibitory effect of a mixture was shown to be a function of the “perchlorate equivalent concentration”, or PEC, defined as the sum of the molar concentrations [$\mu\text{mol/l}$] of each individual inhibitor, each multiplied by the inhibition potency (β) of the inhibitor relative to ClO_4^- (eq. 3 and Table 2). This can be more simply re-written as

$$\text{RAIU} = 1.22 / (1.22 + \text{PEC}),$$

where

$$\text{PEC} = [\text{ClO}_4^-] + [\text{SCN}^-] / 15 + [\text{I}^-] / 30 + [\text{NO}_3^-] / 240.$$

Thus, the PEC for a mixture of RAIU inhibitors represents the molar concentration of ClO_4^- alone that would result in the same amount of inhibition. Responses to mixtures of inhibitors exhibit a coherent concentration-response when plotted as a function of the PEC of each mixture, and that concentration-response curve has the same shape as the concentration-response curve for ClO_4^- alone (Figures 1 – 3).

These results are consistent with a mechanism of simple competition by the four different monovalent anions with similar size for access to the NIS sites on the cell membrane. Estimates from this study of the concentrations corresponding to 50% RAIU inhibition were in the ratios of 1: 15: 30: 240 for ClO_4^- , SCN^- , I^- , and NO_3^- , respectively. These values are in good agreement with the values reported in the literature (as summarized in the introduction) based upon other test systems, including both in vitro and whole animal systems.

A central question in environmental exposure to NIS inhibitors is the modulation of inhibition by dietary iodine. The total iodide uptake is proportional to the amount of I^- present, times the relative uptake, or, based on our model for relative uptake, $[\text{TotalIodideUptake}] = [\text{constant}] \times [\text{I}^-] / (1.22 + \text{PEC})$. This equation predicts that for all levels of iodide nutrition no greater than 1 $\mu\text{mol/l}$ serum, and all possible background levels of the three competing anions, doubling the level of serum iodide will cause an increase in total iodide uptake of between 1.95 and 2.0. Thus, the model of competitive inhibition developed herein predicts that thyroidal I^- uptake is approximately proportional to iodide nutrition for any fixed underlying goitrogen load.

The model does not predict the levels of dietary iodine and goitrogen load at which effects on thyroid economy will occur. We did find one relevant study that looked at the interaction of iodide nutrition and goitrogen (SCN^-) load. In an endemic goiter area in central Africa, 393 neonates, 347 children aged 0 to 7 years and 776 subjects aged 10 to 35 were studied and compared with Belgian controls (46). In approximately half of the neonates, maternal iodide deficiency had been corrected during the 5th month of pregnancy with slowly resorbable iodized oil. With the exception of breast fed infants, cassava was the dietary staple for the population. The authors observed a decrease in goiter prevalence and serum SCN^- among breast fed infants relative to neonates or weaned infants. The authors reported a significant and progressive increase in goiter prevalence, serum SCN^- and hypothyroidism in the months and years following weaning. The multiple correlation coefficient of urinary I^- and urinary SCN^- (which should correlate with serum concentrations) was highly significant for T4, free T4 and TSH. Clearly, in this study, additional iodine did ameliorate the effect of high goitrogen load.

The model predictions developed in this study can also be utilized to place perspective on health risks associated with environmental exposure to trace amounts of ClO_4^- , SCN^- , and NO_3^- from drinking water sources. Environmental exposure recommendations and standards for water are generally based on a dose metric (mg/kg^- day) and then translated into drinking water concentrations assuming that a 70 kg adult drinks 2 liters of water daily. After accounting for differences in the molecular weights of the different anions, in terms of serum concentration on a weight basis, the relative IUI potencies of ClO_4^- relative to SCN^- and NO_3^- are 9 and 150, respectively. To consider relative potencies on an

ingested-weight basis, the serum half lives (approximately 8 hours, 6 days, and 5 hours for ClO_4^- , SCN^- , and NO_3^- , respectively) become important because SCN^- stays in the serum 18-29 times longer than the other anions. In terms of ingested weight basis, the IUI potencies of ClO_4^- relative to SCN^- and NO_3^- are thus calculated to be 0.5 and 240 respectively.

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