



## Exploring the destiny and distribution of thiocyanate in the water-soil-plant system and the potential impacts on human health



R. Calderón<sup>a,\*</sup>, C. Jara<sup>b</sup>, F. Albornoz<sup>c</sup>, P. Palma<sup>d</sup>, N. Arancibia-Miranda<sup>e,f</sup>, R. Karthikraj<sup>g</sup>, K. Manquian-Cerda<sup>e,f</sup>, P. Mejias<sup>h</sup>

<sup>a</sup> Center for Research in Natural Resources and Sustainability, Universidad Bernardo O'Higgins, Fabrica 1990, Segundo Piso, Santiago, Chile

<sup>b</sup> Department of Chemistry, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago de Chile, Chile

<sup>c</sup> Department of Plant Sciences, Faculty of Agronomy and Forest Engineering, Pontificia Universidad Católica de Chile, Avenida Vicuña Mackenna 4860, Macul, Santiago, Chile

<sup>d</sup> Public, Environmental and Labor Health Laboratory, Regional Ministerial Service, Ministry of Health, Metropolitan Region, Santiago, Chile

<sup>e</sup> Faculty of Chemistry and Biology, Universidad de Santiago de Chile, USACH, Casilla 40, C.P. 33, Santiago 9170022, Chile

<sup>f</sup> Center for the Development of Nanoscience and Nanotechnology, CEDENNA, Santiago 16 9170124, Chile

<sup>g</sup> Wadsworth Center, New York State Department of Health, Albany, NY 12201-0509, United States

<sup>h</sup> Department of Biological and Chemical Sciences, Faculty of Natural Resources, Universidad Católica de Temuco, Temuco, Chile

### HIGHLIGHTS

- We evaluated thiocyanate capture and accumulation in chard and spinach.
- Thiocyanate accumulation depended on the treatment and culture time.
- Chard accumulates more thiocyanate than spinach (T2 > T1): leaves > total aerial fraction
- EDI values (0–12 months) for spinach exceeded chronic and subchronic reference doses.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

Editor: Jay Gan

#### Keywords:

Chard  
Spinach  
Uptake  
Thiocyanate  
Food safety  
Intake  
Rhodanide

### ABSTRACT

Endocrine disruptors like thiocyanate are some of the principal causes of chronic disorders worldwide. Prenatal and postnatal exposure to thiocyanate can interfere with normal neurological development in both fetuses and newborns. Currently, little information regarding thiocyanate levels and potential sources of exposure is available. In this study, we evaluated thiocyanate uptake and accumulation in chard and spinach grown under greenhouse conditions. Both chard and spinach are commonly used to produce baby foods. Three thiocyanate concentrations were compared: Control, T1 (30 ng mL<sup>-1</sup>), and T2 (70 ng mL<sup>-1</sup>). Thiocyanate accumulation depended on the concentration and exposure time. Chard was found to accumulate more thiocyanate than spinach, with leaf accumulation > stem accumulation ( $p < 0.0194$ ) and maximum concentrations of 76 ng g<sup>-1</sup> (control), 112 ng g<sup>-1</sup> (T1), and 134 ng g<sup>-1</sup> (T2). The estimated daily intake (EDI) of thiocyanate for chard and spinach (fresh) exceeded the subchronic reference dose of 200 ng<sup>-1</sup> kg<sup>-1</sup> day<sup>-1</sup> and the chronic reference dose of 600 ng<sup>-1</sup> kg<sup>-1</sup> day<sup>-1</sup>. In addition, the EDI of thiocyanate for spinach in baby food exceeded twice the chronic reference dose in the vulnerable newborn–1 year age group.

**Abbreviations:** BAF, bioaccumulation factor; ESI-MS/MS, electrospray ionization tandem mass spectrometry; EDI, estimated daily intake; FCID, Food Commodity Intake Database; LOD, limits of detection; LOAEL, lowest observed adverse effect level; NO<sub>3</sub><sup>-</sup>, nitrate; ND, not detected; ClO<sub>4</sub><sup>-</sup>, perchlorate; HSCN, thiocyanic acid; SCN<sup>-</sup>, thiocyanate; TF, translocation factor.

\* Corresponding author at: Center for Research in Natural Resources and Sustainability, Universidad Bernardo O'Higgins, Fábrica 1990, Segundo Piso, Santiago, Chile.

E-mail address: [raul.calderon@ubo.cl](mailto:raul.calderon@ubo.cl) (R. Calderón).

<http://dx.doi.org/10.1016/j.scitotenv.2022.155502>

Received 10 February 2022; Received in revised form 19 April 2022; Accepted 20 April 2022

Available online 28 April 2022

However, all EDIs were lower than the lowest observed adverse effect level (LOAEL) of  $1.9 \times 10^5 \text{ ng kg}^{-1} \text{ day}^{-1}$ . Further studies are needed that increase our knowledge of thiocyanate levels and potential environmental sources to reduce opportunities for exposure, especially in vulnerable groups.

## 1. Introduction

In recent decades, the rapid expansion of industrial development, agricultural production, and human activities has intensified pollutant inputs into the environment, elevating the risks to human health (Yilmaz et al., 2019). Endocrine disruptors are pollutants of great interest to the international scientific community due to their ability to interfere with the human endocrine system and metabolism (Metcalf et al., 2022). The World Health Organization (WHO) has estimated that ~750 million people worldwide have some type of thyroid pathology, with 60% of this population being unaware of the problem.

Endocrine disruptors, such as perchlorate ( $\text{ClO}_4^-$ ), nitrate ( $\text{NO}_3^-$ ), and thiocyanate ( $\text{SCN}^-$ ), are pollutants that competitively inhibit the transport of sodium iodide to the thyroid gland, resulting in a reduction in hormone (e.g., T3 and T4) production, particularly in people with low levels of iodide consumption (Zhu et al., 2019; Lee et al., 2017; Qin et al., 2014). Thus, pregnant women, newborns, and infants are also particularly at risk of the effects of endocrine disruptors (Zhu et al., 2019; Qin et al., 2014). Currently, one of the main problems associated with child nutrition is estimating the risks associated with the consumption of contaminated foods leading to disease generation (Pivovarov et al., 2016; Mielech et al., 2021).

The dietary intake of vegetables, such as those of the Brassica genus like broccoli, kale, Chinese cabbage, and cauliflower, has been associated with various health promoting effects (Wagner et al., 2013). However, some glucosinolates in Brassica vegetables produce indolylic isothiocyanates with anticarcinogenic activity. In contrast, progoitrin and indolylic glucosinolates degrade to goitrin and thiocyanate, respectively, and may decrease thyroid hormone production (Felker et al., 2016). Thus, it is important to identify potential environmental sources of harmful substances to reduce opportunities for exposure, especially in vulnerable groups.

Thiocyanate is the anion derived from thiocyanic acid (HSCN) and is widely used when manufacturing electronic products and furniture (Laurberg et al., 2009; Jain, 2016). Thiocyanate salts are characterized by high water solubility ( $\text{KSCN}$ :  $>1000 \text{ g L}^{-1}$ ) that is lower than those of other endocrine disruptors, such as perchlorate salts ( $2090 \text{ g L}^{-1}$ ). Although the dynamics of perchlorate in the environment have been investigated, those of thiocyanate have not yet been elucidated (Calderon et al., 2020, 2021). The main route of human exposure to thiocyanate is the metabolization of cyanide from cigarette smoke into thiocyanate in the liver (Willemin and Lumen, 2017; Knight et al., 2018). However, other potential sources of thiocyanate exposure, such as drinking water and agricultural products, remain understudied despite being considered primary sources of exposure to other contaminants (Blount et al., 2008; Leung et al., 2012; Qin et al., 2014; Jain, 2016; Zhu et al., 2019). Soil fumigation is used to control soil-borne pathogens, nematodes, and weeds (Dungan et al., 2003; Zheng et al., 2004), and metam-sodium, a soil fumigant, is in use in several countries for this purpose in quantities ranging from 50 to  $80 \text{ g ha}^{-1}$ . Methyl isothiocyanate (MITC) is the primary breakdown product of metam-sodium (Dungan et al., 2003; Zhang et al., 2005). MITC is a highly volatile compound with broad, non-selective biocidal activity (DPR, 2016). The hydrolysis rate of MITC is low in water but increases significantly upon the addition of sediments similar to those found in bodies of water (DPR, 2002). Due to the relatively low leaching potential and fast degradation of MITC in the presence of water with sediments, the risk of groundwater contamination is low (Tomlin, 2000).

Once absorbed, thiocyanate has a mean half-life of 1–2 weeks and is excreted in the urine (Scherer, 2006). Studies have shown that thiocyanate and perchlorate levels in urine and serum have negative additive effects on normal thyroid gland functioning in pregnant women, newborns, and infants, which constitutes a health risk for these groups (Blount et al., 2009;

Pearce et al., 2012; Leung et al., 2012; Charatcharoenwitthaya et al., 2014; Horton et al., 2015; Lee et al., 2017; Knight et al., 2018). During the transition between the newborn (newborn–1 year) and infant (2–3 years) stages, babies are mainly fed breast milk and processed baby foods that are often made with vegetables, such as spinach, chard, and carrots. Previous studies have shown that spinach and chard accumulate soluble contaminants like perchlorate that can form part of the final food product (Calderon et al., 2021). Currently, no regulations exist for thiocyanate in either baby foods or other processed foods, although regulations are in place for perchlorate (EFSA, 2017). Our previous research (unpublished results) shows that thiocyanate is a common contaminant in various matrices 107 in Chile, such as drinking water ( $1.4\text{--}15.2 \text{ ng mL}^{-1}$ ), irrigation water ( $7.2\text{--}13.7 \text{ ng mL}^{-1}$ ), fruits ( $0.11\text{--}0.56 \text{ ng g}^{-1}$ ), vegetables ( $0.25\text{--}0.74 \text{ ng g}^{-1}$ ), agricultural soils ( $7.2\text{--}125 \text{ ng g}^{-1}$ ), 109 and, fertilizers ( $142\text{--}1132 \text{ ng g}^{-1}$ ). Depending on the characteristics of the soil, irrigation water, and agronomic management at regional levels, a potential risk of the continual incorporation of thiocyanate into the food chain through contaminated crops is present when considering global agricultural production (Zhang et al., 2020). Given this scenario, it is vitally important to study thiocyanate dynamics in the water-soil-plant system in controlled production systems, such as those of greenhouses, in which the conditions (e.g., soil, substrate, or water treatment system characteristics) can be modified to limit pollutant accumulation (Valloton et al., 2017; Calderon et al., 2021; Calderón et al., 2022).

Thus, the objectives of the study were to 1) estimate the uptake, accumulation, and translocation of thiocyanate in the different organs of chard and spinach grown in greenhouses under three treatments and 2) estimate the risk associated with thiocyanate intake in different age groups due to the consumption of chard and spinach. The results of our research may be used by the international scientific community to propose regulations for thiocyanate in food products, particularly baby food, and are thus much needed.

## 2. Materials and methods

### 2.1. Study site

The study was conducted during summer–autumn 2019 at the Faculty of Agronomy and Forestry Engineering of the Pontificia Universidad Católica de Chile in Santiago, Chile.

### 2.2. Greenhouse experiment

The experimental design followed that of Calderon et al. (2020). Briefly, spinach and chard seeds were germinated in seedling trays that had been previously filled with a moist substrate mixture composed of peat and perlite and irrigated daily for 21 days. Subsequently, the seedlings were transplanted into 4-L pots filled with the same substrate mixture. In total, 108 pots were established (54 with chard and 54 with spinach) and assigned to one of three treatments: Control, T1 ( $30 \text{ ng mL}^{-1}$  of thiocyanate), and T2 ( $70 \text{ ng mL}^{-1}$  of thiocyanate). The thiocyanate concentrations were prepared from a standard thiocyanate solution. All experimental treatments were carried out in triplicate, and the pots were distributed in the greenhouse in a completely random design. Each treatment was applied through a drip irrigation system. Each container was supplied daily with 270 mL, administered via four irrigations (2 min) with one dripper ( $2 \text{ L h}^{-1}$ ). The nutrient solution for each treatment was individually mixed in a 120-L container and completely replaced every two weeks. During the first two weeks of the experiment, the nutrient solution was composed of potassium nitrate ( $120 \text{ mg L}^{-1}$ ), calcium nitrate ( $240 \text{ mg L}^{-1}$ ),

monoammonium phosphate (30 mg L<sup>-1</sup>), magnesium sulfate (120 mg L<sup>-1</sup>), and Fe-EDDHA (5 mg L<sup>-1</sup>; Basafer Plus; Compo Expert, Münster, Germany). The pH range of the solution was adjusted to remain between 5.5 and 6.0. At the start of the third week of growth, the fertilizer concentration in the nutrient solution was doubled.

To evaluate the thiocyanate dynamics of the water-soil-plant system, the bottoms of the experimental pots were perforated to collect leachate on a daily basis. Each week, substrate, root, stem, leaf, total aerial fraction (stem + root), and leachate samples were collected and stored at 4 °C until further analysis. The harvest periods corresponded to weeks 1–2 (groups 1–4), 3–4 (groups 2–5), and 5–6 (groups 3–6). Additionally, the thiocyanate levels in the nutrient solution and fertilizer samples were determined.

### 2.3. Sample preparation, extraction, and analysis

Briefly, spinach and chard samples consisting of approximately 0.20 ± 0.02 g of the root, stem, and total aerial fraction were placed in propylene tubes that had been previously fortified with 100 ng mL<sup>-1</sup> of potassium thiocyanate (<sup>13</sup>C, 95–99%). Acetonitrile and acetic acid were added to a final volume of 10 mL. The samples were shaken, centrifuged, and extracted using Envi-Carb cartridges (500 mg, 6 mL; Waters Corporation; Milford, MA, USA). The collected extracts were stored at 4 °C until analysis. Substrate samples (0.15 ± 0.05 g) were combined with Milli-Q water and liquid chromatography grade ethanol, fortified with 100 ng mL<sup>-1</sup> potassium thiocyanate (<sup>13</sup>C, 95–99%), extracted with Envi-Carb cartridges (500 mg, 6 mL; Waters Corporation), and stored at 4 °C. The leachate samples (0.2 mL) were diluted 1000-fold, fortified with 100 ng mL<sup>-1</sup> of potassium thiocyanate (<sup>13</sup>C, 95–99%), shaken, filtered (0.2-µm mesh), and stored at 4 °C. The nutrient solution and fertilizer samples were subjected to the same procedure as the leachate samples.

The thiocyanate concentration was determined using an Agilent 1100 series LC liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) coupled to an API 2000 electrospray ionization tandem mass spectrometry (ESI-MS/MS) triple quadrupole mass spectrometer (Applied Biosystem, Foster City, CA, USA) in negative ionization mode. The mobile phase was composed of methylamine (40 mM) in isocratic mode at a flow rate of 0.3 mL min<sup>-1</sup> using an IonPac AS-21 anion exchange column (250 mm × 2 mm; Dionex, Sunnyvale, CA, USA). Thiocyanate quantification was conducted with an 8-point calibration curve (0–2500 ng mL<sup>-1</sup>) with a linear regression coefficient of 0.99. Blank, duplicate, and spiked samples (100 ng mL<sup>-1</sup>) were included to evaluate the performance of the chromatography system. Thiocyanate was not detected in any of the quality controls. The limits of detection (LOD) were 1 ng mL<sup>-1</sup> for the leachate and nutrient solutions and 6 ng g<sup>-1</sup> for the substrate and plant tissue samples.

### 2.4. Bioaccumulation factor (BAF) and translocation factor (TF)

The bioaccumulation factor (BAF) is used to estimate the capacity of a plant species to accumulate contaminants in its tissues. A BAF > 1 indicates contaminant accumulation, whereas a BAF < 1 indicates the opposite. The translocation factor (TF) is used to describe the ability of a plant species to translocate contaminants from its roots to leaves. A TF > 1 indicates contaminant accumulation in the leaves, whereas a TF < 1 indicates that accumulation occurs in the roots (Calderón et al., 2022).

### 2.5. Risk estimation

The estimated daily intake (EDI) for each age group (newborn–1 year, 2–3 years, 4–10 years, 11–21 years, 22–45 years, and 46–70 years) was determined based on the average thiocyanate concentration (ng g<sup>-1</sup>) in chard and spinach at the harvest stage in each treatment multiplied by the average daily vegetable intake (g) for each age group and divided by the average body mass (kg) for each age group. Information on vegetable consumption rates was obtained from the Food Commodity Intake Database (FCID 2005–2010) of the United States.

## 3. Results and discussion

### 3.1. General characterization

The first evidence of the presence of thiocyanate in fertilizers to date is presented in Table 1. Concentrations from not detected (ND)–397 ng g<sup>-1</sup> were quantified in the nitrogenous, potassic, phosphoric, and magnesium fertilizers used in the study. Interestingly, Chilean fertilizers (i.e., nitrogenous and potassic) are characterized by containing trace amounts of chemical species with no nutritional value, such as perchlorate (Calderón et al., 2022). Both perchlorate and thiocyanate are highly soluble, and the co-existence of these compounds in fertilizers (e.g., magnesium sulfate, potassium nitrate, and calcium nitrate) presents a potential human health risk due to the ingestion of contaminated foods. Monoammonium phosphate, a fertilizer imported into Chile, contained the highest concentration of thiocyanate (397 ng g<sup>-1</sup>).

Our results allow us to hypothesize that agricultural soils worldwide may be potentially contaminated with traces of thiocyanate. Thus, it is necessary to understand thiocyanate dynamics in the water-soil-plant system. In our study, thiocyanate was not detected in substrate, irrigation water, or seedling samples during initial germination. However, in the Control treatment, the nutrient solution contained 10 ng mL<sup>-1</sup> of thiocyanate, which was only due to fertilizer addition.

### 3.2. Uptake and accumulation in tissues

#### 3.2.1. Roots and stems

Soluble contaminant accumulation in leaves is a function of evapotranspiration and the genotype of the plant (Seyfferth and Parker, 2007). In our study, thiocyanate accumulation in the roots increased as a function of time and treatment (T2 > T1 > Control; Fig. 1). The thiocyanate concentration applied in the T2 treatment was 10-fold higher than the concentrations in water samples in Chile (7.2–13.7 ng mL<sup>-1</sup>; median: 7.5 ng mL<sup>-1</sup>). Thiocyanate was not detected in the leachate and substrate samples of either chard or spinach, indicating that thiocyanate was taken up by the roots and translocated to higher organs. As such, a microbial degradation process may be at work within the system, although further research is needed to confirm this hypothesis. A similar tendency to that observed in the roots (Control) was present in the stems. However, an inverse effect was observed in chard (T1), which has a higher capacity to accumulate thiocyanate than spinach (Control; week 6). In T2, spinach accumulated more thiocyanate than chard (week 4–5), although the thiocyanate concentration in chard (85 ng g<sup>-1</sup>) was higher than that in spinach (57 ng g<sup>-1</sup>) at week 6. In addition, a significant difference was present in thiocyanate accumulation between the roots and stems in chard ( $p < 0.0001$ ).

#### 3.2.2. Total aerial fraction and leaves

The thiocyanate accumulation in the leaves and total aerial fraction are presented in Fig. 2. A similar pattern to that observed in the roots and stems occurred in the total aerial fraction and leaves, with thiocyanate accumulation increasing over time. Interestingly, in the Control treatment, the total aerial fraction of chard was found to accumulate twice as much thiocyanate as that of spinach from weeks 3–5. However, these concentrations were the same at harvest. For T1 and T2, thiocyanate accumulation in the total aerial fraction of chard was always higher than that of spinach. The thiocyanate

**Table 1**  
Thiocyanate (SCN<sup>-</sup>) concentrations in fertilizers.

Fertilizer	Concentration of SCN <sup>-</sup> (ng g <sup>-1</sup> )
Magnesium sulphate (MgSO <sub>4</sub> )	354
Basafer (Fe-EDDHA)	N.D.
Potassium nitrate (KNO <sub>3</sub> )	358
Monoammonium Phosphate (NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> )	397
Calcium nitrate (Ca(NO <sub>3</sub> ) <sub>2</sub> )	142

N.D.: Not detected.

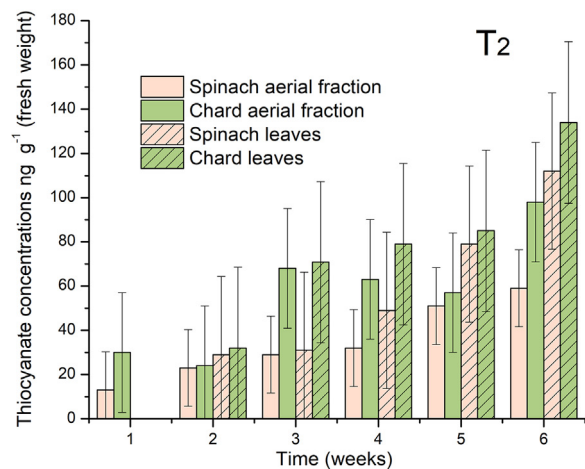
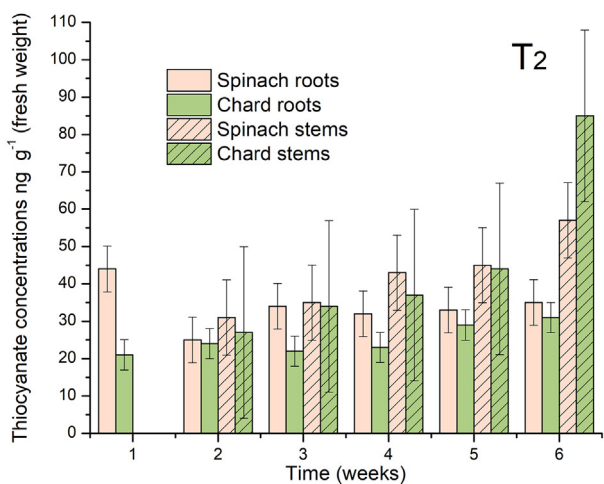
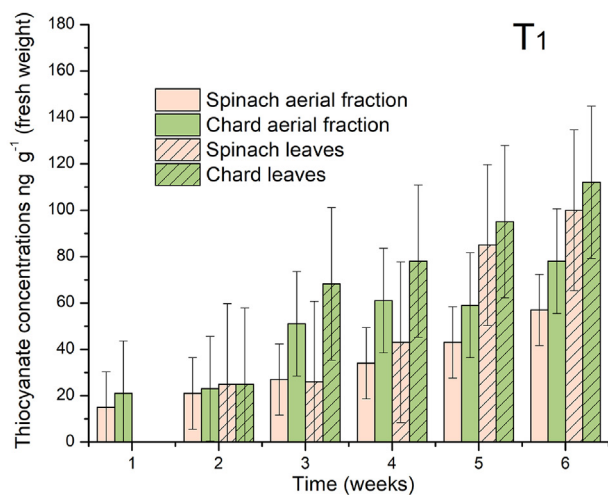
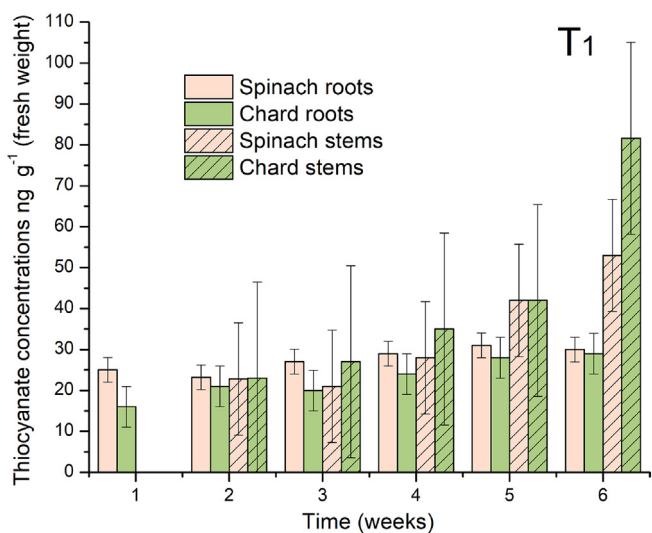
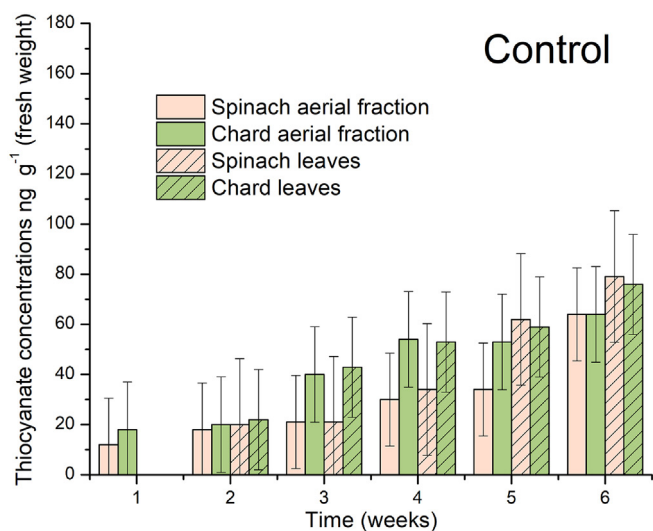
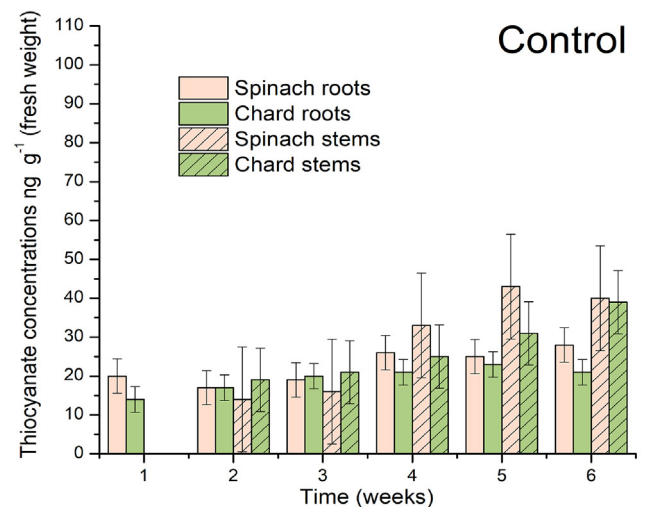


Fig. 1. Distribution of the thiocyanate concentration in the roots and stems of chard and spinach plants in the control, T1, and T2 treatments.

Fig. 2. Distribution of the thiocyanate concentration in the leaves and total aerial fractions of chard and spinach plants in the control, T1, and T2 treatments.

concentrations at harvest for chard in T1 and T2 were 78 and 98 ng g<sup>-1</sup>, respectively.

When comparing thiocyanate accumulation in this study with the perchlorate accumulation in greenhouse crops of Calderón et al. (2022), notable differences are present. Calderón et al. (2022) found that perchlorate accumulation was always higher in spinach in treatments with 1 and 10 mg L<sup>-1</sup> and exceeded the permitted limits for perchlorate in baby foods (20 ng g<sup>-1</sup>) (EFSA, 2017). In our study, thiocyanate accumulation in chard was significantly ( $p < 0.0194$ ) higher than that of spinach, and thus accumulation is a function of the plant species.

In general, the volume and surface area of chard leaves are greater than those of spinach, and thus chard leaves have greater evapotranspiration potential, with the concentrations of soluble contaminants, such as thiocyanate, increasing over time. In a previous study, it was reported that the accumulation of thiocyanate in rice seedlings fertilized with NO<sub>3</sub><sup>-</sup> was higher 4 than that of NH<sup>+</sup> (Zhang et al., 2020). In the Control treatment, thiocyanate accumulation in chard leaves was higher than that of spinach leaves until week 4. However, from week 4 until harvest, the thiocyanate concentration in spinach leaves surpassed that of chard leaves. In T1 and T2, thiocyanate accumulation in chard was always greater than in spinach. The maximum thiocyanate concentrations in chard and spinach in T1 and T2 were 112 and 134 ng g<sup>-1</sup> and 100 and 112 ng g<sup>-1</sup>, respectively.

The results of the statistical analyses show that there were significant differences in thiocyanate accumulation in both the leaves and stems of chard ( $p < 0.0001$ ) and spinach ( $p < 0.0005$ ). The BAF for both species >1, indicating that thiocyanate accumulates in both species (leaf > stem > root). In addition, the TF values indicated that thiocyanate is preferentially translocated from the roots to the leaves in both chard and spinach. Finally, significant differences were present between treatments for chard ( $p < 0.0121$ ) but not for spinach ( $p < 0.0936$ ).

Although thiocyanate accumulation in this study was high, without regulations it is not possible to determine if these types of vegetables represent a potential danger to human health. For example, Sanchez et al. (2008) reported elevated thiocyanate levels in Brassica sp. (1965–56,219 µg kg<sup>-1</sup>). Additionally, Han and Kwon (2009) reported that the average thiocyanate intake (16.3 µmol SCN<sup>-</sup> day<sup>-1</sup> person<sup>-1</sup>) through Brassicaceae vegetables in Korea was lower than the dose required producing adverse effects. In this context, Bhattacharjee et al. (2012) suggest that the high thiocyanate content in both Brassica and non-Brassica vegetables (16.5–42.3 mg kg<sup>-1</sup>) may be in part responsible for the persistence of the endemic goiter in people of the sub-Himalayan Tarai region. New studies are needed that generate baseline data to establish regulations for thiocyanate concentrations in fruits and vegetables to protect consumer health, especially those of vulnerable groups.

### 3.3. Human exposure

Table 2 shows the EDI values calculated by age group for chard and spinach. For chard, the highest EDI values were found in the age groups of 4–10 and 22–45 years, with upper limits of 281 and

348 ng kg<sup>-1</sup> day<sup>-1</sup> for T2, respectively, and 235 and 291 ng kg<sup>-1</sup> day<sup>-1</sup> for T1, respectively. These upper limits exceed the subchronic reference dose of 200 ng kg<sup>-1</sup> day<sup>-1</sup> (EPA, 2012). In the Control treatment, the EDI values did not exceed the subchronic reference dose of 200 ng kg<sup>-1</sup> day<sup>-1</sup> (EPA, 2012).

The EDI calculations were made for spinach considering both the consumption of fresh spinach and spinach in baby foods. In fresh spinach, the EDI values for the age groups spanning 2 to 70 years exceeded the chronic reference dose of 600 ng kg<sup>-1</sup> day<sup>-1</sup>. The highest EDI values of 813 (Control), 1030 (T1), and 1154 (T2) ng kg<sup>-1</sup> day<sup>-1</sup> were present in the age group of 2–3 years. The EDI values for spinach consumption in baby foods were estimated up to 3 years of age. The EDI values for the age group of newborn–1 year were greater than 1000 ng kg<sup>-1</sup> day<sup>-1</sup>, whereas the EDI values for the age group of 2–3 years exceeded the subchronic reference dose but not the chronic reference dose.

In summary, the EDI values in this study are less than the lowest observed adverse effect level (LOAEL), which is  $1.9 \times 10^5$  ng kg<sup>-1</sup> day<sup>-1</sup> (EPA, 2012). However, our results show that there is a potential risk to human health from the consumption of spinach and chard in different age groups, primarily in newborns and infants up to three years of age. Further studies are needed to elucidate the real impacts of thiocyanate intake on human health due to the consumption of contaminated foods.

## 4. Conclusions

Our study is the first to evaluate the uptake and accumulation of thiocyanate in chard and spinach grown in greenhouses. The results show that thiocyanate is a common contaminant in nitrogenous, potassic, and phosphoric fertilizers on the order of ng g<sup>-1</sup>. The thiocyanate accumulation increased over time and depended on the treatment (T2 > T1 > Control), with chard accumulating more thiocyanate than spinach ( $p < 0.0194$ ). Interestingly, statistically significant differences ( $p < 0.0001$ ) were present with regard to thiocyanate accumulation between chard and spinach (leaf > total aerial fraction). The EDI values for chard (Control) exceeded the subchronic reference dose of 200 ng kg<sup>-1</sup> day<sup>-1</sup> (EPA, 2012). For fresh spinach, the EDI values exceeded the chronic reference dose of 600 ng kg<sup>-1</sup> day<sup>-1</sup> for the age groups spanning 2 to 70 years. For spinach in baby foods, the EDI values for the age group of newborn–1 year were higher than 1000 ng kg<sup>-1</sup> day<sup>-1</sup> and exceeded the chronic reference dose, although lower EDI values of ~500 ng kg<sup>-1</sup> day<sup>-1</sup> were found for the age group of 2–3 years. In general, the EDIs were lower than the LOAEL of  $1.9 \times 10^5$  ng kg<sup>-1</sup> day<sup>-1</sup> (EPA, 2012).

## Funding

This study was supported by the National Fund for Scientific and Technological Development (FONDECYT) Program [FONDECYT 11160581] of the National Commission for Scientific and Technological Research (CONICYT), Chile.

**Table 2**

Estimated daily intake (EDI) of thiocyanate (SCN<sup>-</sup>) in chard and spinach for different age groups.

Age group (years)	Chard				Spinach (fresh)				Spinach (baby food)			
	C/BM	EDI Control: 76 ng g <sup>-1</sup>	EDI T1: 112 ng g <sup>-1</sup>	EDI T2: 134 ng g <sup>-1</sup>	C/BM	EDI Control: 79 ng g <sup>-1</sup>	EDI T1: 100 ng g <sup>-1</sup>	EDI T2: 112 ng g <sup>-1</sup>	C/BM	EDI Control: 79 ng g <sup>-1</sup>	EDI T1: 100 ng g <sup>-1</sup>	EDI T2: 112 ng g <sup>-1</sup>
Newborn–1	0	0	0	0	2.3	181.7	230	257.6	12.4	979.6	1240	1388.8
2–3	0	0	0	0	10.3	813.7	1030	1153.6	5.2	410.8	520	582.4
4–10	2.1	159.6	235.2	281.4	8.4	663.6	840	940.8	0	0	0	0
11–21	0	0	0	0	5.7	450.3	570	638.4	0	0	0	0
22–45	2.6	197.6	291.2	348.4	9.6	758.4	960	1075.2	0	0	0	0
46–70	1.3	98.8	145.6	174.2	6.1	481.9	610	683.2	0	0	0	0

EDI in (ng kg<sup>-1</sup> day<sup>-1</sup>).

C/BM: Consumption (g)/Body Mass (kg).

## CRedit authorship contribution statement

**R. Calderón:** Funding acquisition, Writing – original draft, Writing – review & editing, Formal analysis, Resources. **C. Jara:** Formal analysis, Investigation, Methodology. **F. Albornoz:** Formal analysis, Supervision, Writing – review & editing. **P. Palma:** Formal analysis, Writing – original draft, Writing – review & editing. **N. Arancibia-Miranda:** Formal analysis, Investigation. **R. Karthikraj:** Formal analysis. **K. Manquian-Cerda:** Formal analysis. **P. Mejias:** Formal analysis.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Raul Calderon Araya reports financial support was provided by National Commission for Scientific and Technological Research. Raul Calderon Araya reports a relationship with National Commission for Scientific and Technological Research that includes: funding grants.

## References

- Bhattacharjee, A., Chandra, A.K., Malik, T., 2012. Goitrogenic content of common vegetables in Sub-Himalayan Tarai region of eastern Uttar Pradesh. *Int. J. Med. Health Sci.* 1, 32–38.
- Blount, B.C., Ozpinar, A., Alwis, K.U., Caudill, S.P., Gillespie, J.R., 2008. Perchlorate, nitrate, thiocyanate and iodide levels in chicken feed, water, and eggs from three farms. *J. Agric. Food Chem.* 56, 10709–10715.
- Blount, B.C., Rich, D.Q., Valentin-Blasini, L., Lashley, S., Ananth, C.V., Murphy, E., Smulian, J.C., Spain, B.J., Barr, D.B., Ledoux, T., Hore, P., Robson, M., 2009. Perinatal exposure to perchlorate, thiocyanate, and nitrate in New Jersey mothers and newborns. *Environ. Sci. Technol.* 43, 7543–7549.
- Calderon, R., Palma, P., Arancibia-Miranda, N., Un-Jung, K., Silva-Moreno, E., Kannan, K., 2020. Occurrence, distribution, and dynamics of perchlorate in soil, water, fertilizers, vegetables and fruits and associated human exposure in Chile. *Environ. Geochem. Health* <https://doi.org/10.1007/s10653-020-00680-6>.
- Calderon, R., Eltit, K., Wang, Y., Palma, P., Silva-Moreno, E., Arancibia-Miranda, N., 2021. Field study on the uptake, accumulation, and risk assessment of perchlorate in a soil-chard/spinach system: impact of the agronomic practices and fertilization. *\*Sci. Total Environ.* 719, 137411.
- Calderón, R., Jara, C., Albornoz, F., Palma, P., Arancibia-Miranda, N., Karthikraj, R., Zhu, H., 2022. Comparative uptake, translocation, and accumulation of perchlorate between spinach and chard under greenhouse conditions. *\*Food Chem.* 370, 131101.
- Charatcharoenwithaya, N., Ongphiphadhanakul, B., Pearce, E.N., Somprasit, Ch., Chanthasenanont, A., He, X., Chailurkit, L., Braverman, L.E., 2014. The association between perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant Thai women. *J. Clin. Endocrinol. Metab.* 99 (7), 2365–2371.
- DPR. Department of Pesticide Regulation, 2002. Evaluation of Methyl Isothiocyanate as a Toxic Air Contaminant Part A – Environmental Fate. Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.
- DPR. Department of Pesticide Regulation, 2016. Correlating agricultural use with ambient air concentrations of methyl isothiocyanate during the period of 2011-2014. Available from: [https://www.cdpr.ca.gov/docs/emon/airnit/methyl\\_isothiocyanate.pdf](https://www.cdpr.ca.gov/docs/emon/airnit/methyl_isothiocyanate.pdf).
- Dungan, R.S., Gan, J., Yates, S.R., 2003. Accelerated degradation of methyl isothiocyanate in soil. *Water Air Soil Pollut.* 142, 299–310.
- Environmental Protection Agency (EPA), 2012. Provisional Peer-Reviewed Toxicity Values for Thiocyanic acid. Available from: <https://cfpub.epa.gov/ncea/prprt/documents/ThiocyanicAcid.pdf>.
- European Food Safety Authority (EFSA), 2017. Dietary exposure assessment to perchlorate in the European population. Available from: <https://www.efsa.europa.eu/en/efsajournal/pub/5043>.
- Felker, P., Bunch, R., Leung, A.M., 2016. Concentrations of thiocyanate and goitritin in human plasma, their precursor concentrations in brassica vegetables, and associated potential risk for hypothyroidism. *Nutr. Rev.* 74, 248–258.
- Han, H., Kwon, H., 2009. Estimated dietary intake of thiocyanate from Brassicaceae family in Korean diet. *J. Toxicol. Environ. Health A* 72, 1380–1387.
- Horton, M.K., Blount, B.C., Valentin-Blasini, L., Wapner, R., Whyatt, R., Gennings, Ch., Factor-Litvak, P., 2015. Cooccurring exposure to perchlorate, nitrate and thiocyanate alters thyroid function in healthy pregnant women. *Environ. Res.* 143, 1–9.
- Jain, R.B., 2016. Trends and variability in the levels of urinary thiocyanate, perchlorate, and nitrate by age, gender, race/ethnicity, smoking status, and exposure to environmental tobacco smoke over 2005–2012. *Sci. Total Environ.* 557–558, 221–230.
- Knight, B.A., Shields, B.M., He, S., Pearce, E.N., Braverman, L.E., Sturley, R., Vaidya, B., 2018. Effect of perchlorate and thiocyanate exposure on thyroid function of pregnant women from south-west England: a cohort study. *Thyroid. Res.* 11, 9.
- Laurberg, P., Pedersen, I.B., Carlé, A., Andersen, S., 2009. The relationship between thiocyanate and iodine. *Comprehensive Handbook of Iodine*, pp. 275–281 <https://doi.org/10.1016/b978-0-12-374135-6.00028-5>.
- Lee, S.Y., McCarthy, A.M., Stohl, H., Ibrahim, S., Jeong, C., Braverman, L.E., Ma, W., He, X., Mestman, J.H., Schuller, K.E., Jahreis, K.A., Pearce, E.N., Leung, A.M., 2017. Urinary iodine, perchlorate, and thiocyanate concentrations in U.S. lactating women. *Thyroid* 27 (12), 1574–1580.
- Leung, A.M., Braverman, L.E., He, X., Schuller, K.E., Roussilhes, A., Jahreis, K.A., Pearce, E.N., 2012. Environmental perchlorate and thiocyanate exposure and infant serum thyroid function. *Thyroid* 22 (9), 938–943.
- Metcalfe, C.D., Bayen, S., Desrosiers, M., Munoz, G., Sauve, S., Yargeau, V., 2022. Methods for the analysis of endocrine disrupting chemicals in selected environmental matrices. *Environ. Res.* <https://doi.org/10.1016/j.envres.2021.112616>.
- Mielech, A., Púscion-Jakubik, A., Socha, K., 2021. Assessment of the risk of contamination of food for infants and toddlers. *Nutrients* 13 (7), 2358.
- Pearce, E.N., Alexiou, M., Koukkout, E., Braverman, L.E., He, X., Alevizaki, M., Markou, K.B., Iliast, L., 2012. Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women from Greece. *Clinical Endocrinology* 77, 471–474.
- Pivovarov, Y.P., Milushkina, O.Y., Tikhonova, I., Aksenova, O.I., Kalinovskaya, M.V., 2016. Chemical pollution of baby food products in the Russian Federation. *Gigiena i Santirajia* 95, 707–711.
- Qin, X., Zhang, T., Gan, Z., Sun, H., 2014. Spatial distribution of perchlorate, iodide and thiocyanate in the aquatic environment of Tianjin, China: environmental source analysis. *Chemosphere* 111, 201–208.
- Sanchez, Ch.A., Blount, B.C., Valentin-Blasini, Krieger, 2008. Perchlorate, thiocyanate, and nitrate in edible cole crops (*Brassica* sp.) produced in the lower Colorado river region. *Bull. Environ. Contam. Toxicol.* 79, 655–659.
- Scherer, G., 2006. Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke. *Exp. Toxicol. Pathol.* 58 (2–3), 101–124.
- Seyfferth, A.L., Parker, D., 2007. Effects of genotype and transpiration rate on the uptake and accumulation of perchlorate (ClO<sub>4</sub><sup>-</sup>) in lettuce. *Environ. Sci. Technol.* 41, 3361–3367.
- Tomlin, C.D.S., 2000. The Pesticide Manual. 12th edition. British Crop Protection Council, United Kingdom.
- Valloton, A., Strawn, L.K., Latimer, J., 2017. Guide to identifying food safety hazards in greenhouse systems. Available from (2017):Virginia Cooperative Extension. [https://www.pubs.ext.vt.edu/content/dam/pubs\\_ext\\_vt\\_edu/HORT/hort-254/HORT-254.pdf](https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/HORT/hort-254/HORT-254.pdf).
- Wagner, A.E., Terschluessen, A.M., Rimbach, G., 2013. Health promoting effects of brassica-derived phytochemicals: from chemopreventive and anti-inflammatory activities to epigenetic regulation. *Oxidative Med. Cell. Longev.* 964539. <https://doi.org/10.1155/2013/964539>.
- Willemin, M.E., Lumen, A., 2017. Thiocyanate: a review and evaluation of the kinetics and the modes of action for thyroid hormone perturbations. *Crit. Rev. Toxicol.* 47, 537–563.
- Yilmaz, B., Terekeci, H., Sandal, S., Kelestimur, F., 2019. Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Rev. Endocr. Metab. Disord.* 21, 127–147.
- Zhang, Y., Spokas, K., Wang, D., 2005. Degradation of methyl isothiocyanate and chloropicrin in forest nursery soils. *J. Environ. Qual.* 34, 1566–1572.
- Zhang, Q., Feng, Y., Yu, X., Zhang, H., Liang, Y., 2020. Effects of nitrogen fertilization on removal kinetics of thiocyanate (SCN<sup>-</sup>) in rice seedlings. *Int. J. Environ. Sci. Technol.* 17, 4291–4298.
- Zheng, W., Yates, S.R., Papiernik, S.K., Guo, M., 2004. Effect of Combined Application of Methyl Isothiocyanate and Chloropicrin on Their Transformation. 33, pp. 2157–2164.
- Zhu, F., Huang, M., Jiao, J., Zhuang, P., Mao, L., Zhang, Y., 2019. Environmental exposure to perchlorate, nitrate, and thiocyanate in relation to obesity: a population-base study. *Environ. Int.* 133, 105191.