INDIVIDUAL-LEVEL HETEROGENEITY IN ENVIRONMENTAL RISK ASSESSMENT MODELS

by

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CHAPTER I

Introduction

1.1 Introduction and Motivation

Risk assessments have driven public health decisions in the United States since 1975, when the EPA completed its first risk assessment document [98]. By 1983, the National Research Council had set up the formal risk assessment framework of hazard identification, exposure assessment, dose response assessment, and risk characterization that is still in use today[23]. This system remains a dominant public policy tool for evaluating public health concerns, informing regulatory and technological decisions, and prioritizing research needs and funding.

Though useful, the process of risk assessment is by no means perfect. One of the greatest hurdles with risk assessments is their ability to deal with variability. Standard quantitative risk assessment models generally use mean values for parameters, without accounting for the variability that arises from uncertainty and heterogeneity in these values. However, when parameter uncertainty is included, quantitative microbial risk assessment (QMRA) models have provided considerably more variable ranges in their output [26], potentially reflecting true differences in risk. Neglecting such variability in parameter inputs, therefore, may lead to erroneous decisions for public health and risk management.

When standard statistical tests of heterogeneity are used, they generally do not account for specific study characteristics including differences in individuals, populations, exposure characteristics, and methods [135]. Multiple computational and methodological adjustments have been made to the risk assessment process in an attempt to overcome this issue. Some have advocated use of Bayesian approaches. While naturally encompassing uncertainty[78], these approaches are complex in their interpretation, requiring an understanding of probabilistic representations of uncertainty and the use of prior information. The EPA uses a much simpler system, dealing with differences in exposure by requiring consideration of possible exposure levels: central tendency and high end. The interpretation of this method is much simpler, but may miss trends and dynamics that are not incorporated in only these two values.

Risks that arise from the environment are inherently complex due to the interplay of biological, ecological, and social factors that result in a variety of exposure and transmission scenarios. While risk assessment models of environmental risks will always encompass a great deal of uncertainty, systematic, quantitative assessment of heterogeneity can expose underlying variability arising from both natural processes and methodological sources. This variability not only can affect overall risk estimates, but also can result in disparate effects of an environmental hazard or disease. Understanding both the sources and effects of underlying heterogeneity in environmental risks allows for more accurate risk estimates while identifying those populations that are most vulnerable

In this work, I demonstrate the importance of incorporating sources of heterogeneity in risk assessment, and provide a perspective on how this heterogeneity can be integrated in a way that is tractable yet does not ignore important trends. This

is done through the examination of three scenarios of environmentally acquired hazards. 1) individual exposure estimates for dioxins, furans, and PCBs; 2) population outbreak risk of norovirus; and 3) population infection risk of dengue.

Aim 1

Identify the effect of individual heterogeneity on the excretion rates of dioxins, furans, and PCBs, and provide a method of half-life estimation that incorporates individual characteristics.

Background and Significance

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are lipophilic chemicals that can persist in the body for years [106]. An individuals body burden is a product of multiple years of exposure [94] and a lifetime of varying elimination rates. Different congeners of dioxins, furans, and PCBs each have different persistence in the human body, reflected in their different reported half-lives. The apparent half-life, defined as the change in concentration in the body over time, is the net result of elimination from the body, changes in body composition, and intake from the environment. For each congener, variation in half-life exists both among individuals, and within the same individual over his or her lifetime. This variability can be partially attributed to personal characteristics including age, body fat, smoking status, and breastfeeding. The factors that affect elimination rates must be taken into account when predicting past exposures and body burdens of these chemicals, and when comparing current serum congener profiles to exposure media.

Innovation

Although studies show an association between individual characteristics and the pharmacokinetics of dioxins, furans, and PCBs in the human body, there is no standard method for determining a chemical′s half-life as a function of these factors. The majority of half-life studies for dioxins, furans, and PCBs follow accidental or occupational exposures, and no single study exists covering the lifespan of people with varying physical characteristics. Despite summaries of pharmacokinetic data of dioxins, furans [89], and PCBs [74], exposure and body burden estimation has been hindered by the absence of a half-life range and value for each congener.

This study provides congener-specific reference half-life values for adults and infants as well as a method to modify this value based on individual characteristics, resulting in a method of individual half-life estimation. Based on a literature search, values are defined that approximate the half-life for 29 PCDD, PCDF, and PCB congeners in infants and adults. The relationships between half-life and individual characteristics are examined, and an equation is presented that uses the chosen reference values to predict half-lives based on these individual characteristics.

Aim 2

Determine a realistic distribution of post infection norovirus shedding duration and demonstrate how incorporation of heterogeneity in this parameter can affect estimates of outbreak risk.

Background and Significance

Norovirus is the most common cause of epidemic gastroenteritis across all age groups [31], responsible for >90% of viral gastroenteritis and about 50% of allcause outbreaks worldwide [92]. In the United States, norovirus causes an estimated 71,000

hospitalizations annually, costing nearly 500 million dollars per year [72]. These infections typically occur during explosive and short-lived epidemics, followed by inter-epidemic periods of prolonged weak transmission [11]. This epidemiological pattern is due in part to its low infectious dose [123], high strain diversity, high environmental stability, and lack of lasting immunity [38].

Another characteristic that affects norovirus epidemiological dynamics is long-term asymptomatic viral shedding. A typical norovirus infection is self-limiting, with the infectious period lasting a few weeks, but some individuals can shed norovirus long past their symptomatic period [35, 99, 132]. These long-shedders may affect epidemiological dynamics by acting like viral reservoirs, keeping the disease in the population longer, potentially past the length of naturally acquired immunity. They may also act as incubators, creating antigentically diverse variants of the virus that may be sufficiently different from circulating strains to spark novel outbreaks.

A better understanding of the risk factors for persistent viral shedding can lead to the *a priori* identification of long-shedding individuals, information that can be used in norovirus outbreak interventions and prevention. More stringent guidelines for those who work with these individuals, as well as interventions targeted at mitigating the effect of potential long-shedders who do become infected, could reduce population risk while protecting more sensitive populations from outbreak recurrence.

Innovation

The presence of a long-shedding group has been shown to impact outbreak dynamics and population outbreak risk in models of salmonella in cattle [63], but such effects have not been shown in humans. Models of human norovirus transmission often assume a fast rate of recovery. For example, of two recent models of nosocomial norovirus transmission, one assumes an infectious period of less than 2 days

[132], and the other a distribution from 1 to 8 days [65], though shedding has been recorded for much longer. To date, we know of no norovirus transmission models that incorporate long-shedding. Therefore, the effect of long-shedders on norovirus transmission risk remains largely unknown.

In this work we create a more realistic empirical distribution of norovirus shedding duration by extracting data from the literature. This more realistic distributions is then used to inform a series of norovirus transmission models. We use these models to examine how the presence of a long-shedding group affects population risk, including the probability, outcome, and severity of norovirus transmission. We show that long-shedders can increase these transmission outcomes and should therefore be included when estimating norovirus risk.

Aim 3

Identify differences in dengue infection and show how these differences can be partially explained by heterogeneity in rates of travel and road access.

Background and Signficance

Dengue stands as one of the greatest contemporary challenges to public health. Its human and economic costs have increased dramatically over the last few decades as it has spread around the world. The global expansion of its range and incidence is reflected most strikingly in the Americas and the Caribbean, where urbanization, insufficient political commitment, increased travel, and inadequate resources have contributed to the re-emergence and increase of dengue. This has occurred in all of the 19 countries previously certified to be free of its vector, *Aedes aegypti* [122]. In 2008, more than 900,000 cases of dengue and 25,000 cases of dengue haemorrhagic fever (DHF) were reported in the Americas [91], and the geographical reach of dengue

virus transmission now spreads from southern USA to Northern Argentina.

Dengue has generally been considered an urban disease, but is spreading to rural areas in many countries. It is unknown what favors transmission in these settings [24]. Lack of spatial clustering supports the hypothesis that there is little autochthonous transmission in rural areas [24]. This indicates that repeated introductions from human migration and travel may be responsible for infections in these regions. Regression studies have shown that migration was a predictor of the presence of dengue IgG antibodies at baseline and subsequent seroconversion to dengue [24]. Previous studies indicate a link between road access and increased risk of diarrhoeal disease [29] and malaria [133], and dengue vector studies have found that mosquitoes are distributed along roadways [28], demonstrating that road access may play a role in its spread. The construction of a new road into a rural area in northern coastal Ecuador provided an opportunity to undertake a natural experiment that analyzes the effects that differences in road access have on dengue infection.

Innovation

Dengue IgG antibodies are thought to provide lifelong immunity to a particular strain, theoretically remaining in the body for a lifetime. Cross-sectional analysis of the presence of these antibodies provides data spanning over 50 years, and can be used to understand dengue transmission over time. In 1996 a road was constructed connecting the region to the coast and to the Andes, increasing migration rates and travel. Age and location linked seroprevalence data can show how differences in road access affect rates of infection over time.

In this work, we will use field collected longitudinal and cross-sectional serological data to examine how heterogeneity in road access can affect dengue risk. Specifically, we examine the effect of road access on dengue risk, and explore possible causes for

differences in infection distribution including changes in levels of travel to areas of known high dengue transmission. By understanding how heterogeneity in road access and travel is linked to differences in risk, we can both identify regions that are at risk for dengue introduction, and inform appropriate scales for intervention.

CHAPTER II

Apparent Half-Lives of Dioxins, Furans, and PCBs as a Function of Age, Body Fat, Smoking Status, and Breastfeeding

2.1 Abstract

This study reviews the half-life data in the literature for the 29 dioxin, furan, and PCB congeners named in the World Health Organization Toxic Equivalency Factor scheme, with the aim of providing a reference value for the half-life of each congener in the human body and a method of half-life estimation that accounts for an individual's personal characteristics. Data from more than 30 studies containing congener-specific elimination rates were compared. Half-life data were extracted and compiled into a summary table. A subset of these data was created based on defined exclusionary criteria. Values for each congener are defined that approximate the half-life in an infant and in an adult. A linear interpolation of these values is used to examine the relationship between half-life and age, percent body fat, and absolute body fat. Predictive equations were developed based on these relationships and adjustments for individual characteristics. The half-life of dioxins in the body can be predicted using a linear relationship with age that is adjusted for body fat, smoking, and breastfeeding. An alternative method based on a linear relationship between half-life and total body fat is suggested, but this approach requires further

testing and validation with individual measurements.

2.2 Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are lipophilic and can persist in the body for years [106]. An individual′s body burden is a product of multiple years of exposure [94] and a lifetime of varying elimination rates. Different congeners of dioxins, furans, and PCBs each have different persistence in the human body, reflected in their different reported half-lives. The apparent half-life, defined as the change in concentration in the body over time, is the net result of elimination from the body, changes in body composition, and intake from the environment. For each congener, variation in half-life exists both among individuals and within the same individual over his or her lifetime. This variability can be partially attributed to personal characteristics including age, body fat, smoking status, and breastfeeding. The factors that affect elimination rates must be taken into account when predicting past exposures and body burdens of these chemicals, and when comparing current serum congener profiles to exposure media.

2.2.1 Relationship with Age

The relationship between age and half-life is complex, as age is strongly associated with other factors that affect half-life length (e.g., smoking status and percent body fat). Age may have an independent effect through an age-related reduction in hepatic elimination capacity [8], and as humans age they generally experience an increase in and a redistribution of body fat as well as a relative change in organ sizes, causing a redistribution of lipophilic chemicals that also greatly alters their rates of elimination [131]. This relationship is further muddied by a strong cohort effect that is seen in cross-sectional studies, caused by varying levels of persistent chemicals in the

environment. During the 1960s and 1970s, environmental levels of dioxins were much higher than they are today, leading to higher body burdens of the more persistent congeners in older people, above the level expected from persistence alone [94].

In a study of German chemical workers, half-life length of numerous dioxins and furans was positively associated with increasing age [32]. This is consistent with a study on the Yusho and Yucheng cohorts of penta-, hexa-, and hepta-CDF half-lives [68]. Studies on the Ranch Hand cohort show a slight negative association [137] or no association [79], but this may be due to the narrow age range characterizing these cohorts. Studies with child or infant subjects report significantly shorter half-life values than studies with adult cohorts [59, 67, 68]. In children under 18 exposed in the incident in Seveso, Italy, a strong association between half-life and age was found, and children had significantly shorter half-lives than adults [52].

The rapid growth of neonates and children, especially in lipid stores, can result in a dramatically reduced apparent half-life through dilution [20]. However, the effect of dilution alone is not sufficient to create the observed reduction in apparent half-life; it may also be due to a faster metabolism, an increased rate of fecal lipid excretion, or a combination of these events [2, 54]. As children age, their rate of growth slows and the effect of elimination on apparent half-life becomes more important than that of dilution.

2.2.2 Relationship with Smoking Status

Smoking has been associated with lower levels of dioxins and dioxin-like compounds. Active smokers have lower PCDD, PCDF, and PCB serum levels than both non-smokers and passive smokers [16, 9], and levels of dioxin-like PCBs in human milk are negatively related to the smoking habits of the mothers [127]. This is in agreement with Flesch-Janys et al. [32], who observed that the half-

lives of some PCDD and PCDF congeners appeared to be dependent on smoking status. A significantly faster decay was observed in smokers, with increases ranging from 30% (2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)) to 100% (1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin (HxCDD)). Smoking induces the transcription of cytochrome P450 (CYP) 1A2 and other enzymes responsible for the elimination of dioxin and dioxin-like compounds, most likely through the activation of the Ah- receptor by polycyclic aromatic hydrocarbons in tobacco smoke [143]. The total effect of smoking on half-life may be through this increased induction of dioxin degrading enzymes, or through a combination of other physiological effects.

2.2.3 Relationship with Body Burden

Dioxins are known to up-regulate the enzymes responsible for their own elimination, meaning that high concentration could increase elimination rates. Modeled and experimental data in rats show that at high exposures the induction of CYP1A2 is a more important factor for persistence in the body than differences in adipose tissue distribution [30]. A concentration dependent biphasic elimination rate has been identified in cases of acute poisoning [1], in the Seveso incident [8, 80], in children [52], and in the Yusho and Yucheng rice oil poisonings [68, 101], where the rate at high concentrations is much higher than that at lower concentrations. Human data suggest that the serum concentration where this transition occurs is at 700 ppt [52] for TCDD, and at 1000 - 3000 ppt for PCDFs [68]. These concentrations are considerably higher than those measured in people exposed to present background levels.

2.2.4 Relationship with Body Fat

Dioxins, furans, and PCBs are highly lipophilic, and therefore partition preferentially in adipose tissue and other body fat. High amounts of adipose tissue, estimated by body mass index (BMI), are associated with higher serum levels of dioxins and furans [22], where $BMI = \frac{weight(kg)}{height^2(m)}$. Because it acts as a reservoir for these chemicals, increases in adipose tissue results in their storage rather than transportation to excretory and metabolizing organs. Models based on rat data demonstrate a linear relationship between increasing fat mass and half-life length at low body burdens, with the impact of adipose tissue on half-life becoming less important at high body burdens [30], due to the relationship with concentration described above.

The relationship between percent body fat and half-life is apparent throughout the Ranch Hand study [82, 79, 81], but these studies did not find a significant relationship between half-life and short-term changes in percent body fat. These findings are supported by the German occupational cohort, where a one percent increase in percent body fat was associated with a decay rate decrease in the range of 0.0031 ng/kg/yr (1,2,3,6,7,8-HxCDD) to 0.0063 ng/kg/yr (1,2,3,4,6,7,8–hepta-chlorinated dibenzo-p-dioxin (HpCDD)) for dioxins, and about 0.005 ng/kg/yr for furans [32]. This study did show an increased decay rate in workers with intermediate weight loss, but in a limited number of people ($n = 3$). Half-life is moderately correlated with both BMI and body fat mass in children, but longitudinal data from children are difficult to interpret due to their fast growth and simultaneous age-related changes [52].

2.2.5 Relationship with Breastfeeding

For women, lactation can be the major route of elimination of many persistent lipophilic chemicals [2, 108]. Twenty percent or more of the maternal body burden of some persistent pollutants, such as PCBs, can be transferred during six months of lactation [62, 87]. The reduction of half-life due to breastfeeding is both congener specific and duration dependent. The amount of fat in breast milk varies over time, affecting the partitioning of chemicals from the body [21]. Different congeners partition differently into the breast milk from the blood [108, 107] and this distribution is thought to be dependent on the molecular weight of the congener. Along with molecule diameter and differences in lipophilicity, molecular weight may influence membrane permeability, thus causing differences in distribution [136].

Although studies show an association between individual characteristics and the pharmacokinetics of dioxins, furans, and PCBs in the human body, there is no standard method for determining a chemical′s half-life as a function of these factors. The majority of half-life studies for dioxins, furans, and PCBs follow accidental or occupational exposures, and no single study exists covering the lifespan of people with varying physical characteristics. Despite summaries of pharmacokinetic data of dioxins, furans [89], and PCBs [74], exposure and body burden estimation has been hindered by the absence of a half-life range and value for each congener. This study provides congener specific reference half-life values for adults and infants and a method of half-life estimation based on individual characteristics. Based on a literature search, values are defined that approximate the half-life for 29 selected PCDD, PCDF, and PCB congeners in infants and adults. The relationships between

half-life and individual characteristics are examined, and an equation is presented that uses the chosen reference values to predict half-lives based on these individual characteristics.

2.3 Methods

An extensive literature search was conducted for human half-life or decay values for the 29 congeners of dioxins, furans, and dioxin-like PCBs included in the World Health Organization 2005 Toxic Equivalency Factor (TEF) scheme [129]. Measured or modeled half-life values for each congener and the age of the subject or mean age of the cohort were recorded from over 30 studies (Tables 2.1, 2.2, and 2.3).

A subset of data was selected based on the following criteria: blood serum concentrations below <700 ppt total TEQ at the time of sampling, adult subjects, and measurements that were not reported as inaccurate in later studies. Half-life values that were calculated assuming steady state conditions were retained if less than 25 years, because this assumption is inappropriate for more persistent substances with significantly higher historical levels. The mean and range of half-lives were calculated for the retained subset to establish a representative set of half-lives for each congener in a moderately exposed adult.

The adult reference values were selected to represent a 40 - 50 year-old with blood dioxin concentrations in the range where the rate of elimination is driven by fat levels. Sources that provided consistent data across congeners and that were within the range of all measured data were preferentially chosen. Infant reference values were chosen to represent an individual under two years old. When infant data were not available, the adult reference value for the congener was multiplied by the ratio of the length of the adult half-life over the infant half-life for TCDD. Half-life

variation as a function of individual characteristics was examined. When the mean age of the cohort was not explicitly provided, the mean age at the mid-point of sampling was estimated. When percent body fat or total body fat data were not available, the mean age-specific BMI reported in the NHANES 2003-2004 study [13] was converted to percent body fat. For adults, the approach proposed by Deurenberg et al. [25] was used:

$$PercentBodyFat = 1.20BMI + 0.23age - 10.8sex - 5.4$$

where sex corresponds to $females = 0$, and $males = 1$.

We used this approach in adults because, unlike the method developed by Knapik et al. [57] that is used by Flesch-Janys et al. [32] and the Ranch Hand cohort analysis [79, 137], it takes into account both age and sex. Studies have shown that if age is not included in the conversion from BMI to percent body fat, it may seriously underestimate percent body fat in older people [25, 45].

In children (ages 0 to 19), a series of age-based equations presented by Hattis et al. [45] are used to predict percent body fat (pbf) for each age in months. Total body fat was estimated by multiplying the average weight reported in the NHANES data for a given age and sex by the calculated percent body fat [13]. Based on the apparent relationships between half-life and these parameters, we propose a procedure of half-life estimation that is a function of age, percent body fat, smoking status, and breastfeeding.

2.4 Results and Discussion

2.4.1 Review of reported half-life values

A comprehensive report of half-life values for dioxins, furans, and PCBs is presented in Tables 2.1, 2.2, and 2.3. Studies that are listed more than once are those

that report multiple half-life values, generally corresponding to measurements on different individuals. Of the studies examined, one third are limited to TCDD: five of these report on the Ranch Hand Cohort [79, 80, 81, 95, 137], three with kinetic data based on the incident in Seveso, Italy [52, 80, 86], one on a poisoning incident in Austria [37], and two based on an adult male volunteer [96, 110]. There are sixteen different measurements based on the Yu-Cheng and Yusho cohorts [17, 50, 67, 68, 102, 103, 101, 114]. Six studies report models or measurements based on occupational exposures [10, 32, 100, 109, 130, 138]. Five studies have information only on infants and children [40, 53, 54, 59, 67, 139], and two data sets are based on general populations [89]. There were an average of 10 reported values for each dioxin and furan congener, but only 4 for each PCB. There were no half-life data for 1,2,3,7,8,9-hexa-chlorinated dibenzofuran (HxCDF).

The ranges of the reported value subsets for adults are shown in Figure 2.1 (dioxins and furans) and Figure 2.2 (PCBs), and the values are shown in Tables 2.1, 2.2, and 2.3. The comparison of reported half-life values reveals large variation across congeners. For example, the mean half-lives of octa-chlorinated dibenzofuran (OCDF), tetra-chlorinated dibenzofuran (TCDF), and 1,2,3,7,8-pentachlorinated dibenzofuran (PeCDF) are all less than three years, whereas the mean half-lives for some of the HxCDD congeners are over a decade. The half-lives in the PCBs range from only a few months (PCB 77) to a few decades (PCB 157), and one study reported a >100 -fold range in metabolic clearance rates between PCB congeners [9].

Within each congener, half-life values reported from the literature vary substantially, typically by a factor 2-3, but up to a factor 35 within the subset. This variation may be a result of different exposure concentrations or scenarios, differences in the demographics of the considered cohort, or differences in the pharmacokinetic model

Study	2378-TCDD	12378-PeCDD	123478-HxCDD	123678-HxCDD	123789-HxCDD	1234678-HpCDD	OCDD
Flesch-Janys et al. 1996 (Median, range)	7.2 (2.5-∞)	15.7(3.6-∞)	8.4 (1.4-∞)	13.1(2.9-∞)	4.9 (2.0-∞)	3.7(1.6-16.1)	6.7 (1.8-∞)
Flesch-Janys et al. 1996 (Parametric Est.)	6.1	11.2	9.8	13.1	5.1	4.9	6.7
Rohde et al. 1999	9.2(5.8-15.4)	13.9(9.9-23.1)	13.9(7.7-19.8)	11.6(4.3-23.1)	7.7(5-9.2)	4.3(2.9-5.8)	8.7(5.8-11.6)
Geusau et al. 2002	1.5*						
Geusau et al. 2002	2.9*						
Gorski et al. 1984				3.5*	3.2*	5.7*	
Leung et al. 2006	0.43*	0.36*		0.44*		0.36*	0.5*
Leung et al. 2006	0.36*	0.28*		0.33*		0.28*	0.42*
Poiger and Schlatter 1986	5.8*						
Schlatter et al. 1991	9.7						
Pirkle et al. 1989	7.1 (5.8-9.6)						
Wolfe et al. 1994	11.3(10.0-14.1)*						
Michalek et al. 1996	8.7 (8.0-9.5)						
Michalek and Tripathi 1999	7.6 (7.0-8.2)						
Michalek et al. 2002	7.5 (4.5-∞)						
Kerger et al. 2006 (<18)	1.6*						
Kerger et al. 2006 (>18)	3.2*						
Michalek et al. 2002 (First 0.27 years)	0.34(0.16-∞)*						
Michalek et al. 2002 (3-16.35 years)	6.9(4.15-∞)						
Needham et al. 1994	7.8						
Kreuzer et al. 1997 (Infant)	0.4*						
Kreuzer et al. 1997 (Adult)	5*						
Ogura 2004 (blood)		6.7(4.9-9.6)		42(29-60)*		5.8(4.0-8.3)	22(18-26)
Ogura 2004 adipose	6.7(3.3-14)	6.6(3.6-12)		24(12-50)	9.2(3.2-27)	1.4(0.7-3.0)	5(1.8-14)
Liem & Theelen 1997	6.2	8.6	19	70*	8.5	6.6	5.6
Ogura 2004 (Liem & Theelen 1997)	7.8	11	12	12	6.8	8.8	5.7
Ogura 2004 (Flesch-Janys et al. 1996)	6.3	8.3	7.8	10	4.6	3.2	4.6

Table 2.1: Congener specific half-life values for dioxins from the literature. Values marked with an asterisk are those that fit exclusionary criteria for the subset. Infinity (∞) is used when at least one person had an increase in serum concentrations between measurements.

Study	2378-TCDF	12378-PeCDF	23478-PeCDF	123478-HxCDF	123678-HxCDF	123789-HxCDF	234678-HxCDF	1234678-HpCDF	1234789-HpCDF	OCDF
Flesch-Janys et al. 1996 (Median, Range)			19.6 (12.6-31.5)	6.2 (1.9-∞)	6 (2.1-∞)	5.8 (3.1-19.8)	3 (2.1-∞)	3.2 (2.1-∞)		
Flesch-Janys et al. 1996 (Parametric Est.)				6.4	7.2		3.1			
Rohde et al. 1999 (Median, Range)			13.9 (4.6-23.1)	8.7 (4.1-17.3)	5.8 (3.6-9.2)	9.9 (8.7-12.6)	3.9 (2.5-4.6)			
Gorski et al. 1984									1.7*	1.8*
Leung et al. 2006			0.23*							
Leung et al. 2006			0.3*							
Schecter et al. 1990 (adipose)			4.7	2.9	3.5		6.5			
Schecter et al. 1990 (blood)			7.2	4.4	4.3		4.1			
Schecter et al. 1990 (both)			4.5	4	4.9		6.8			
Masuda 1995			3.1	3.3			2.4			
Ryan and Masuda 1989		1.7 (1.3-2.9)	2.4 (2.1-5.1)			2.4 (1.6-6.1)				
Ryan et al. 1993 (patient 1)			1.9*	2.1*			2.9*			
Ryan et al. 1993 (patient 2)			2.3	2.9			2			
Ryan et al. 1993 (patient 3)			2.2	2.7			2.1			
Iida et al. 1995			9.1	8.6						
Masuda 2001(Median, Range)			2.9 (2.7-3.6)	3.5 (2.7-3.6)			2.5 (2.2-2.6)			
Kashimoto et al. 1983			1.5	1.5						
Leung et al. 2005 (>3ppb)		1.1*	2.3*			1.5*				
Leung et al. 2005 (<3ppb)		7.5	5.9				3.6			
Leung et al. 2007 (>3ppb)		1.1	2.3			1.5				
Leung et al. 2007 (<3ppb)		7.2	5.7			3.5				
Masuda 2001 (Median, Range)		7.7 (5.2-14.3)	5.1 (3.9-6.9)			3.5 (2.6-6.6)				
Masuda 1995			8.9	5.4			3.9			
Ryan et al. 1993 (Median, Range)			9.6 (5.7-36)	7.8 (4.3-54)						
S Ogura 2004 (blood)			4.9 (3.3 -7.1)	9.9 (6.6-15)	17 (11-26)		4.8 (3.2-7.2)			
Ogura 2004(adipose)	0.2 (0.1-0.4)	0.4 (0.2-1.0)	5 (2.7-9.1)	3.7 (1.3-10)	5.8 (1.4-25)	2.1 (0.8-5.8)	1.4 (0.5-3.8)		2.1 (0.7-6.2)	
Liem & Theelen 1997	0.4	0.9	9.9	5.7	6.2	2.4	2.6		0.2	
Liem & Theelen 1997; Ogura 2004	1.4	2.9	10	7.7	24	3.6	5	10	0.7	
Flesch-Janys et al. 1996	2.4	3.9	7.8	5.6	7.1	3.1	2.8	5.2	1.6	

Table 2.2: Congener specific half-life values for furans from the literature. Values marked with an asterisk are those that fit exclusionary criteria for the subset. Infinity (∞) is used when at least one person had an increase in serum concentrations between measurements.

Study	PCB 77	PCB 81	PCB 126	PCB 169	PCB 105	PCB 114	PCB 118	PCB 123	PCB 156	PCB 157	PCB 167	PCB 189
Masuda 1995							1.7		4.9			
Shirai and Kissel 1996				10.4			1.1		1.62			
Ryan et al. 1993							1.1*		3.3*			
Ryan et al. 1993							1.2		5.4			
Ryan et al. 1993							1.3		4			
Chen et al. 1982					0.56		0.82					
Shirai and Kissel 1996					0.58		0.83		∞			
Shirai and Kissel 1996					0.51		0.77		∞			
Masuda 2001 (Median, Range)							1.6 (1.5 -1.9)		5.3 (3.8-5.6)			
Masuda 2001 (Median, Range)						17.6 (6.9-33.7)		13.2 (8.5-21.5)				
Ryan and Masuda 1991, Masuda 1995						17.6		13.4				
Ryan et al. 1993				10.4								
Brown et al. 1989					3.9		5.8					
Brown and Lawton 2001	5.02		11		7.0	31.7*	10.8	15.3	100*	20*	35*	166.7*
Buhler et al. 1988							0.5*					
Wolff and Schecter 1991										4.6*		
Wolff et al. 1992 (Median, Range)					∞		9.6 (7.4-23)					
Ogura 2004 (blood)			1.6 (1.2-2.1)	7.3 (5.2-10.4)	2.4 (1.7-3.3)	10 (7.4-14.2)	3.8 (2.8-5.3)	7.4 (5.3-10)	16 (11-23)	18 (13-26)	12 (8.7-17)	22 (16-32)
Ogura 2004 (adipose)	0.1	0.7 (0.4-1.2)	2.7 (1.6-4.5)	13 (8.8-19)	2.7 (1.5-4.8)	25 (16-40)*	4.2 (2.3-7.5)	12 (5.8-25)	38 (23-63)*	27 (16-44)*	10 (5.2-19)	41 (24-69)*
Liem & Theelen 1997	0.1		2.7									

Table 2.3: Congener specific half-life values for PCBs from the literature. Values marked with an asterisk are those that fit exclusionary criteria for the subset. Infinity (∞) is used when at least one person had an increase in serum concentrations between measurements.

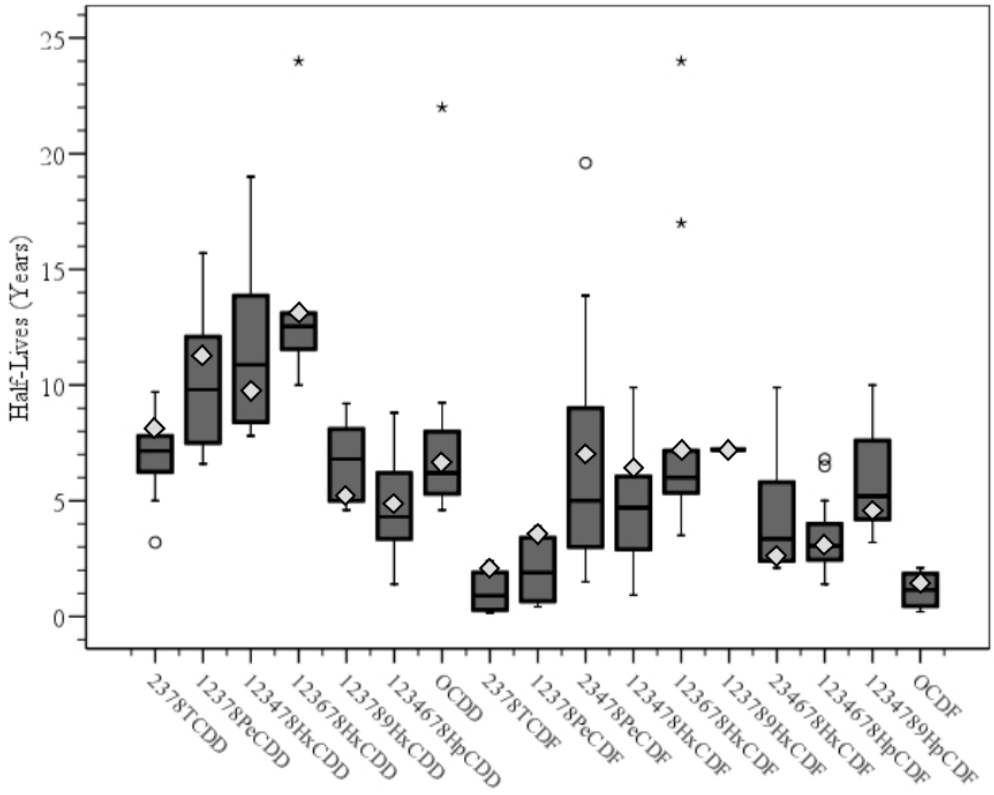


Figure 2.1: Range of half-life values (in years) for dioxins and furans based on a subset of values from the literature. Bars represent 25th, 50th, and 75th percentiles. Diamonds show the placement of the reference values within this range, circles represent individuals that are not outliers, and asterisks show outliers.

used in half-life calculation. Several studies reported on a single person or had very small sample sizes, resulting in unstable mean values. For example, the 15.7-year half-life reported in Flesch-Janys et al.[32] for 1,2,3,7,8-PeCDD becomes 11 years when one worker close to background is excluded. Some of the variability in reported half-life values can be explained through differences in physiological processes among individuals and different congener properties. However, very short half-lives (i.e. <1 year) are unlikely for the most frequently found congeners because of the high exposures required to sustain measurable body burdens, and very long half-lives (>10 years) may be artifacts of ongoing exposures [114].

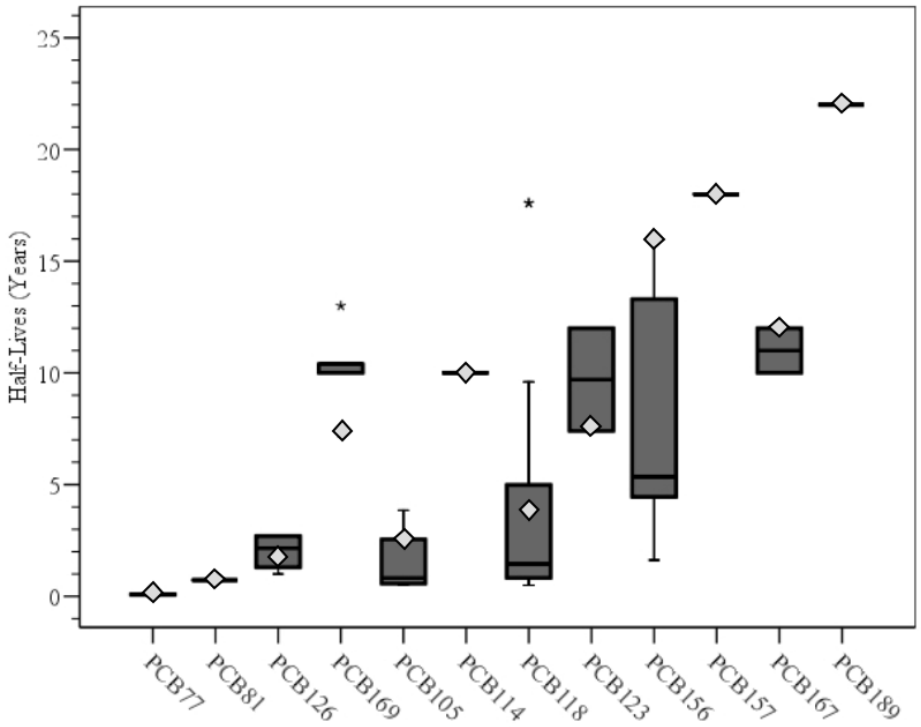


Figure 2.2: Range of half-life values (in years) for PCBs based on a subset of values from the literature. Bars represent 25th, 50th, and 75th percentiles. Diamonds show the placement of the reference values within this range and asterisks show outliers

Most cohorts are composed of adult males exposed to high concentrations, though measurements were sometimes carried out years after exposure. Half-life measurements for persons at or near background levels, including those with no history of substantial exposure or those who have returned to background levels after significant exposure, may be confounded by the effect of probable continuous exposure to background levels of dioxins. Half-life measurements and the influence of other factors (such as smoking and body fat) may be better evaluated when sampled from persons with higher accidental exposures, after accounting for concentration-dependent effects. Most of the studies report concentrations normalized by gram of lipid, and assume a conserved equilibrium between dioxins and lipids across the body. The suitability of this measurement to calculate the overall body burden depends on the distribution of the congeners into adipose tissue. Though different congeners partition differently into different organs [49, 56], a correlation between levels in the blood and levels in adipose tissue is supported [48].

2.4.2 Variation in half-life as a function of age

A positive association between age and half-life is observed Figure 2.3. Though this may indicate a direct relationship between age and half-life, it also incorporates the effect of other parameters, such as age related changes in percent body fat. The influence of body fat, using BMI as a surrogate, is included in the displayed regressions, which use the mean age-specific BMI reported for the 2003-2004 NHANES study [13].

The points representing literature reported data in Figures 2.3 - 2.6 are generally averages of a range of ages and a range of half-life values. These ranges, where available, are presented in Tables 2.1 - 2.3. The non-linear variations seen at low ages when we use the model proposed by Van der Molen et al. [130] are linked

Study	Age	N	Time from exposure (years)	Time of follow up (years)	Cohort	Notes
Flesch-Janys et al. 1996	32-79 Mean =48.7	43	0 - 37 (mean 5.4)	1-9 (mean 5.6)	Occupational	a
Rohde et al. 1999	41-73	6	-	4-6	Occupational	b
Geusau et al. 2002	27, 30	2	0	3	Poisoned Austrian Women	
Gorski et al. 1984	Child	1	-	2.5	Child (wood in home)	c
Leung et al. 2006	Infant	2	0	1	Breast fed Infants	
Schecter et al. 1990	late 50s to early 60s	1	2	3	Occupational	
Poiger and Schlatter 1986	42	1	0	<1	Adult Male Volunteer	
Schlatter 1991	47	1	-	6	Adult Male Volunteer	d
Pirkle et al. 1989	-	36	<10	5	Ranch hand	
Wolfe et al. 1994	31.8-66	337	<10	5	Ranch hand	e
Michalek et al. 1996	31.8-66	213	14.8	10.3	Ranch hand	e
Michalek et al. 1999	31.8-66	97	<9.3	15	Ranch hand	e
Michalek et al. 2002	18-38	97	9-33	15	Ranch hand	f
Kerger et al. 2006	0.5 - 16.6	45	0	17	Seveso	
Kerger et al. 2006	>18	45	0	17	Seveso	
Michalek et al. 2002	16-71	35	0	0.27	Seveso	
Michalek et al. 2002	16-71	54	3	13.35	Seveso	
Needham et al. 1994	-	27	-	-	Seveso	c
Kreuzer et al. 1997	<1	20	0	<1	Infants	
Kreuzer et al. 1997	40	-	-	-	Model based on infants	a
Masuda 1995	25	3	0.6	15	Yu-Cheng	c,g
Ryan and Masuda 1989	-	2-4		-	Yu-Cheng	h
Shirai and Kissel 1996	17-69	19	1-14	8-9	Yu-Cheng and Yusho	i
Ryan et al. 1993	17,25,33	3	1-10	9	Yu-Cheng (Individual)	
Iida et al. 1995	-	7	14	1	Yu-Cheng and Yusho	b,j
Chen et al. 1982	-	17	0	1	Yu-Cheng	
Shirai and Kissel 1996	-	20-24	<1	0.7-4.7	Yu-Cheng	i
Masuda 2001	17-33	3	1	15	Yu-Cheng	
Kashimoto et al. 1983	-	30	<1	1-2	Yu-Cheng	k
Leung et al. 2005	18-80	8	1-14	15	Yu-Cheng and Yusho	
Leung et al. 2007	18-80	8	1-14	15-19	Yu-Cheng and Yusho	
Masuda 2001	31-51	5	14	16	Yusho	
Masuda 1995	-	5	-	-	Yusho	c,g
Ryan et al. 1993	33-69	16	14-22	8	Yusho (5 individuals)	
Brown et al. 1989	-	39	1-26	7.7	Occupational	
Buhler et al. 1988	50	1	<1	<1	Male volunteer	
Wolff and Schecter 1991	2-6	4,5	-	-	Children, contaminated material	m,n
Wolff et al. 1992	45	18-165	<1	3.83	Occupational	
Ogura 2004(blood)	20-65	253	-	-	General Japanese population	
Ogura 2004(adipose)	40 - 59	10	-	-	General Japanese population	
Liem and Theelen 1997	-	-	-	-	General Dutch population	c
Liem and Theelen 1997; Ogura 2004	-	-	-	-	General Dutch population	a,o
Flesch-Janys et al. 1996; Ogura 2004	48.7	-	-	-	Occupational	a,o

Table 2.4: Characteristics and study information for studies with congener specific half-life data. a) Modeled Value b) Fecal Clearance only c) Data accessed from Ogura 2004 d) Data accessed from Flesch-Janys et al. 1996 e) Age in 1982 f) Age during tour of duty g) Also published in Ryan and Masuda 1991 h) Data accessed from U.S. EPA 2003 i) Application of model presented to data from study in Chen et al. 1982 j) Data accessed from Ryan et al. 1993 k) Data accessed from abstract l) Reported two metabolic clearance rates, not apparent half-life values. Clearance rates were assumed to be additive and half-lives were calculated as follows: $t_{1/2} = \frac{1}{k_a} + \frac{1}{k_b}$. m) Did not account for growth, may be near background n) Data accessed from Shirai and Kissel 1996 o) Application of kinetic model to data in parenthesis

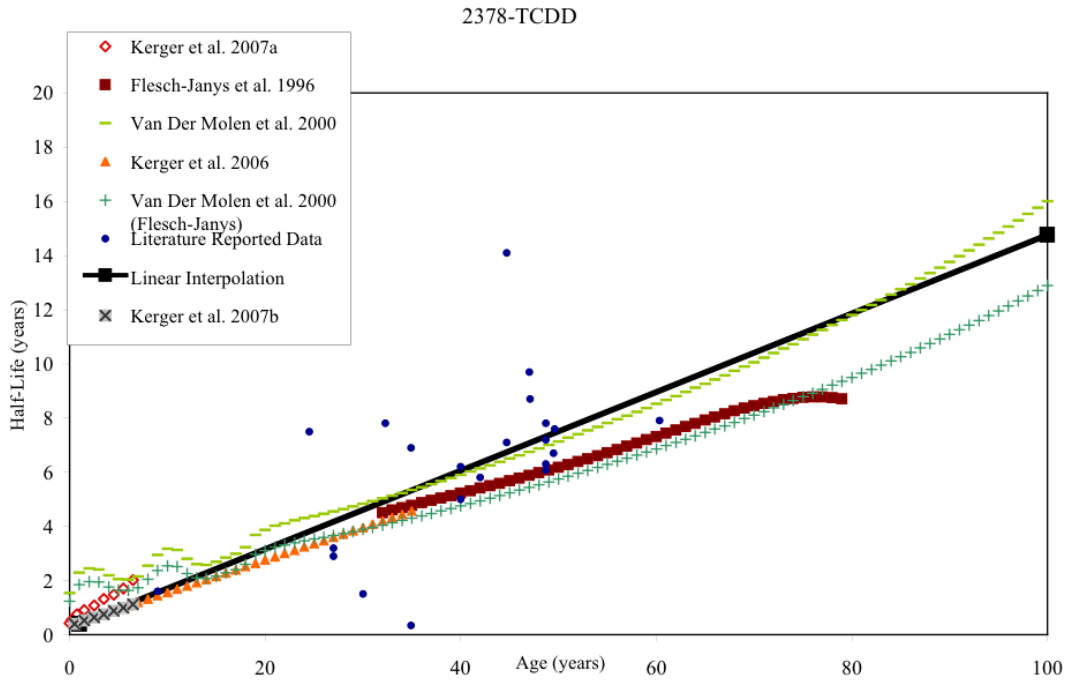


Figure 2.3: Half-Life of 2,3,7,8 TCDD in years as a function of age (in years). Van der Molen et al. (Flesch-Janys) refers to the application of the model presented by Van der Molen et al. (2000) to the Flesch-Janys et al. (1996) data as done by (Ogura 2004). The solid line represents the linear interpolation between the infant and adult reference half-lives (slope and intercept given in Table 5). Literature reported data refers to values from the current literature and presented in Table 2.1.

to modeled variations in body fat during adolescence, but are not confirmed by experimental data.

The Kerger et al. 2006 data correspond to children with concentrations below 700 ppt [52] and support the hypothesis of a close to linear increase in half-life between ages 0 to 35. The slopes calculated with this method were similar to slopes for adults calculated with the method provided by Flesch-Janys et al. [32], spanning adults 30 to 80 years old. However, the equation proposed by Flesch-Janys et al. [32] may be problematic above the age of 60 years old, as very small variations in the elimination rate could lead to substantial divergence in half-live length, as observed in the case of 1,2,3,7,8-PeCDD Figure 2.4.

Overall, we observed a nearly linear association between half-life and age, which is most likely linked to the combined effects of growth-caused dilution at young ages and an increase in body fat at older ages. However, this association does not account for inter-individual variation at each age.

2.4.3 Variation of half-life with body fat

Percent body fat is a good predictor of half-lives in adults as shown for 2,3,7,8-TCDD in Figure 2.5. This method is inappropriate for infants and children (identified by oval in figure), due to drastic changes in percent body fat and short half-lives.

The discrepancy between percent body fat and half-lives observed at young ages suggests the use of absolute body fat mass to account for the effect of fat over the entire age range Figure 2.6. Total body fat was obtained by multiplying calculated percent body fat by age-specific NHANES weight averages [13]. Further data collection is needed to confirm the validity of the relationship between body fat mass and half-life.

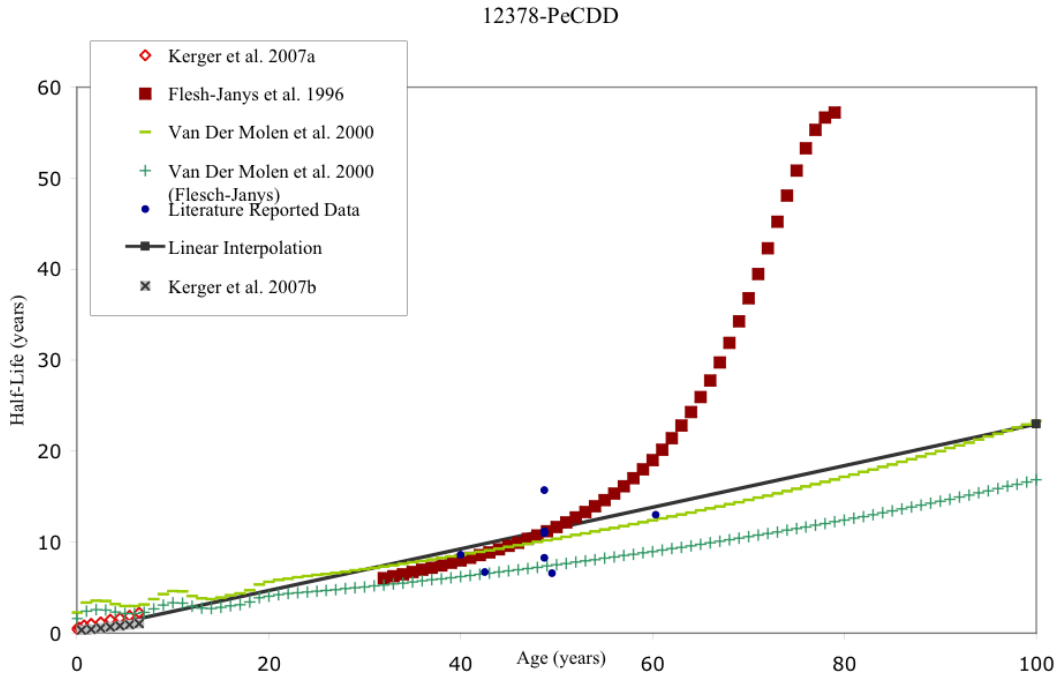


Figure 2.4: Half-life of 12378-PeCDD in years as a function of age in years. Van der Molen et al. (Flesch-Janys) refers to the application of the model presented by Van der Molen et al. to the Flesch-Janys et al. (1996) data (Ogura 2004). Literature reported data refers to values from the current literature and presented in Table 2.1. The solid line represents the linear interpolation between the infant and adult reference half-lives (slope and intercept given in Table 2.5). As shown by the top line, the equation proposed by Flesch-Janys et al. may be problematic above the age of 60 years old, as very small variations in the elimination rate could lead to substantial divergence in half-live length.

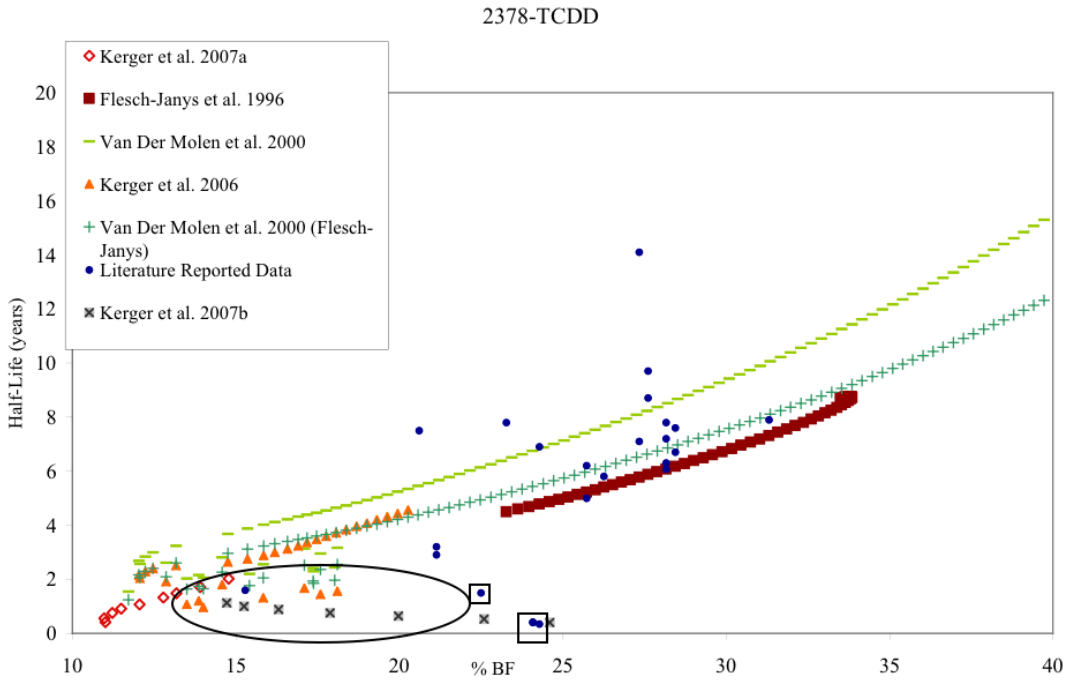


Figure 2.5: 2,3,7,8-TCDD Half-life (in years) as a function of percent body fat. Literature reported data refers to values from the current literature and presented in Table 2.1. The oval shows the area where the relationship of increased half-life with increased body fat does not hold. These values represent young subjects. The squares show subjects whose half-lives were measured when they had serum concentrations that were well above the level of increased induction of degradation enzymes.

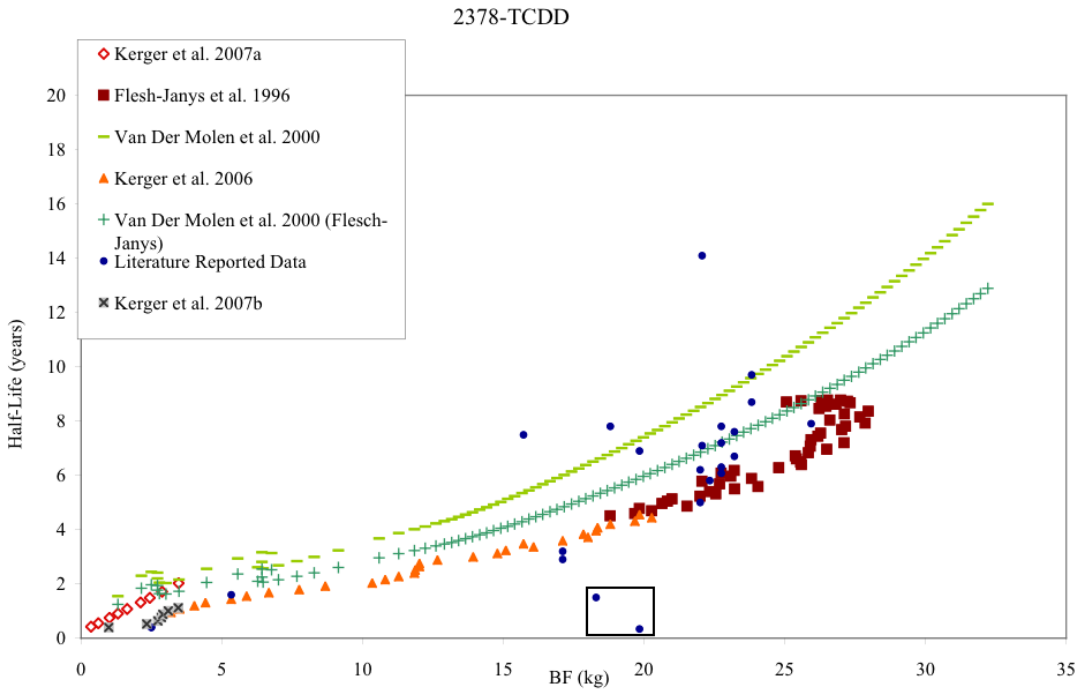


Figure 2.6: 2,3,7,8-TCDD Half-life (in years) as a function of total body fat (in kg). Literature reported data refers to values from the current literature and presented in Table 2.1. The two points below the rest of the data (in square) represent subjects whose half-lives were measured when they had serum concentrations well above the level of increased induction of degradation enzymes.

2.4.4 Reference half-life values

The regression method used by Flesch-Janys et al. [32] was preferentially used for adult reference half-life values because it covers multiple congeners in a consistent way; incorporates information for percent body fat, sex, and smoking status; and the resulting values are within the range of the other values in the literature. In the case of TCDD, the single median value given by Flesch-Janys et al. [32] was used as the reference value, because of its consistency with other reported data. For dioxin and furan congeners not reported in the 1996 Flesch-Janys et al. study, the model proposed by Van der Molen et al. [130] was used to determine a reference half-life, using the median age and percent body fat from Flesch-Janys et al. (48.7 years, 21.9%, respectively). For 1,2,3,7,8,9-HxCDF, which had no available half-life data, the reference half-life for 1,2,3,6,7,8-HxCDF was used.

Reference half-lives of PCB 77 and PCB 81 were based on measurements from samples of adipose tissue, while reference half-lives for the ten remaining PCB congeners were determined based on measurements of blood [89]. These values correspond to half-lives observed in the general Japanese population, assuming steady-state conditions. Because of the large decrease in dioxin, furan, and PCB concentrations in the environment in the last 30 years, the steady-state assumption is only appropriate for congeners with half-lives that are significantly shorter than the time elapsed from the peak in environmental concentrations; the half-lives of more persistent congeners could be over estimated. Reference half-life values for infants were based on congener specific values reported by Leung et al. [67] where available. These values are modeled estimates based on earlier reported concentration data for PCDD and PCDF congeners in breastfed infants [2, 3]. These reference values are based on existing data, and better numbers may be available with the generation of new data. In some

cases it may be appropriate to use the median values, which are also provided in Table 2.5.

2.4.5 Methods for individual half-life calculation

Based on the relationships discussed above, two methods are proposed to predict individualized apparent half-lives of dioxins, furans, and PCBs over a lifetime. We specifically focus on half-lives resulting from moderate levels of exposure, comparable to those resulting from the general exposure of the U.S. population. The use of a simple multi-linear regression model to predict half-life as a function of age and BMI or body fat is problematic because data for age and BMI coefficients are lacking for several congeners, and as previously discussed, percent body fat is not a good predictor of half-lives at young ages.

To overcome these limitations, the first method that we propose is a linear relationship of half-lives with age. The slope ($beta_{age}$) and the intercept ($beta_{0(age)}$) coefficients were found by using a linear interpolation between the infant and adult reference half-lives (given in table 2.5. Inter-individual variation in body composition and smoking habits are accounted for with two multiplicative factors (equation 2.1). The observed linear influence, supported by modeled results [30], of the percent body fat at $age = i$, is incorporated in the calculation by multiplying the original equation by the ratio of the individual percent body fat (pbf_i) to the reference percent body fat for that age ($pbf_{ref(agei)}$). The reference percent body fat was determined by converting the age specific BMI values from the NHANES data to percent body fat using the method proposed by Duerenberg et al. [25] and presented above. Similarly, the effect of smoking is introduced through a unitless multiplicative smoking factor (SF_i). The ratios of the decay rate of smokers to non-smokers in Flesch-Janys et al. [32] are used when available, ranging from 0.5 to 0.7, corresponding to a 50%

	Infant Reference Half-Life (yr)	Adult Reference Half-Life (yr)	Median Half-Life	Reference Adult Age (yr)	Source	Smoking Factor (SF)	Blood-Milk Ratio (K_{BM})	Intercept (β_0)	Slope (β_{age})
2378-TCDD	0.4*	7.2	6.3	48.7	a	0.739	0.92	0.26	0.15
12378-PeCDD	0.3*	11.2	8.5	48.7	b	0.683	1.21	0.09	0.23
123478-HxCDD	0.5	9.8	10.9	48.7	b	0.509	1.44	0.35	0.20
123678-HxCDD	0.4*	13.1	12	48.7	b	0.635	1.32	0.12	0.27
123789-HxCDD	0.3*	5.10	6.8	48.7	b	0.665	1.51	0.18	0.10
1234678-HpCDD	0.3*	4.9	3.7	48.7	b	0.525	1.87	0.22	0.10
OCDD	0.5*	6.7	5.7	48.7	b	0.551	3.3	0.33	0.14
2378-TCDF	0.1	2.1	0.9	48.7	c	0.648	1.1	0.08	0.04
12378-PeCDF	0.2	3.50	1.9	48.7	c	0.648	1.6	0.13	0.07
23478-PeCDF	0.3*	7.0	4.9	48.7	c	0.648	1.15	0.13	0.14
123478-HxCDF	0.4	6.4	4.8	48.7	b	0.692	1.79	0.23	0.13
123678-HxCDF	0.4	7.2	6	48.7	b	0.695	1.91	0.26	0.15
123789-HxCDF	0.4	7.2	-	40.0	d	0.648	1.39*	0.19	0.15
234678-HxCDF	0.2	2.8	3.4	48.7	c	0.648	1.38	0.10	0.06
1234678-HpCDF	0.2	3.1	3	48.7	b	0.832	2.59	0.11	0.06
1234789-HpCDF	0.3	4.6	5.2	48.7	c	0.648	4.28	0.17	0.09
OCDF	0.1	1.4	1.6	48.7	c	0.648	3.4	0.05	0.03
PCB 77	0.0	0.1	0.1	49.5	e	0.648	1.39*	0.00	0.00
PCB 81	0.0	0.7	0.73	49.5	e	0.648	1.39*	0.03	0.01
PCB 126	0.1	1.6	2.7	42.5	f	0.648	0.67	0.05	0.04
PCB 169	0.4	7.3	10.4	42.5	f	0.648	1.24	0.24	0.17
PCB 105	0.1	2.4	2.4	42.5	f	0.648	0.72	0.08	0.06
PCB 114	0.5	10.0	25	42.5	f	0.648	0.69	0.33	0.23
PCB 118	0.2	3.8	1.6	42.5	f	0.648	0.87	0.13	0.09
PCB 123	0.4	7.4	12	42.5	f	0.648	0.52	0.24	0.17
PCB 156	0.9	16.0	5.35	42.5	f	0.648	1.16	0.53	0.37
PCB 157	1.0	18.0	20	42.5	f	0.648	1.26	0.59	0.42
PCB 167	0.7	12.0	12	42.5	f	0.648	1.19	0.39	0.28
PCB 189	1.2	22.0	41	42.5	f	0.648	1.99	0.72	0.51

Table 2.5: Reference half-life values (in years) and model parameters for equations 2 and 3 for dioxins, furans, and PCBs. The infant reference values marked with asterisks were taken from Leung et al. (2006). The Blood to Milk Ratio values marked with asterisks are the geometric mean of all Blood to Milk Ratio values. Sources of adult reference values: a) (Flesch-Janys et al. 1996) median value b) (Flesch- Janys et al. 1996) regression values c) (Van der Molen et al. 2000) d) There were no data for this congener. The half life values were taken to be the same as 1,2,3,6,7,8-HxCDF e) (Ogura 2004) Blood data f) (Ogura 2004) Adipose tissue data

to 30% reduction in half-life (Table 2.5). When not available, the geometric mean of all available smoking factors was used, corresponding to a 35% reduction in half-life. Gender differences are indirectly accounted for by the different percent body fat values for males and females at each age. The predicted half-life (years) for an individual, i, as a function of age, smoking status, and percent body fat i is calculated using the empirical model formalized by equation 2.1.

$$t_{1/2}(age_i, smoke, pbf)_i = [\beta_{0age} + \beta_{age}(age_i)](SF_i)\frac{pbf_i}{pbf_{ref(agei)}} \tag{2.1}$$

This equation estimates half-lives for adults that are comparable to those obtained with the approach proposed by Flesch-Janys et al. 1996 (See Appendix, Figure 1), while extending its applicability to children and adults above 60 years old. A mathematical equation describing the additional rate of elimination due to breastfeeding (equation 2.2) is based on the observed effect of breastfeeding in a cohort of German women [136]. According to this study, a breastfeeding woman expels an estimated 8.76 kg of fat per year through lactation (qf [kg/day] 0.8 kg milk / day of average 3 % lipid), and partition coefficients between blood lipid and milk fat for each congener (K_{BM}, unitless) range from 0.5 to 4.3 (Table 2.5) [136]. Δt_{bfed} (unitless) represents the fraction of the considered year during which the woman was actively breastfeeding, and pbf_i (%) and BW_i (kg) are the woman′s percent body fat and body weight, respectively.

$$k_{bfed} = \frac{q_f * \Delta t_{bfed}}{K_{BM}(\frac{pbf_i}{100})BW_i} \tag{2.2}$$

Assuming no interaction between breastfeeding and the other half-life determinants, the overall predicted apparent half-life for a woman who is actively breast-

feeding is obtained by adding the effect of elimination through breastfeeding to other age, smoking, and body fat adjusted processes (equation 2.3).

$$t_{1/2}(total) = [\frac{1}{t_{1/2}(age, smoke, pbf)} + \frac{k_{bfed}}{ln(2)}]^{-1} \tag{2.3}$$

This method predicts a half-life of 4.3 years for TCDD in a 30 year-old, non-smoking woman with 30% body fat if she did not breastfeed that year, and a half-life of 1.8 years if she breastfed for six months.

The alternative proposed strategy to model congener specific half-lives is based on an observed apparently linear relationship (Figure 2.6) with absolute body fat (kg), and is formalized in equation 2.4. The same correction for smoking status is used as in the first approach (equation 2.1).

$$t_{1/2}(smoke_i, bf_i) = [\beta_{0(bf)} + \beta_{bf}(\beta bfi)]SF_i \tag{2.4}$$

There is insufficient data to test this equation, and this approach requires further data collection and validation.

2.5 Discussion

Reported half-lives of dioxin and dioxin-like congeners in humans vary widely both between and within different dioxin, furan, and PCB congeners. Age, a measure of body fat, smoking habits, and breastfeeding status are strong determinants of the elimination rates observed in humans. This study integrates these critical factors into an empirical model that predicts the half-lives of the 29 WHO TEF scheme congeners over a human lifespan. We support a method of half-life estimation that is a function of age. A nearly linear relationship is shown between half-life and body

fat, but further study and new data are required to evaluate the validity of any estimation methods based on this approach.

Pharmacokinetic information is scarce for many PCB congeners, and many existing studies report on PCB mixtures rather than individual congeners. Further, much of the existing data does not take into account the effect of ongoing exposures to background levels. The half-life range and reference values may be refined as more congener specific data becomes available. Pharmacokinetic studies across multiple congeners, which take into consideration demographic factors, are necessary to determine accurate elimination rates. Further study into the causes of inter- and intra-individual elimination rate variability, such as the effect of genetic polymorphisms and sensitivity to known factors, would further refine half-life estimation accuracy.

The equations described here represent a simple and relatively consistent approach that can be used to determine individual apparent half-lives for numerous dioxin, furan, and PCB congeners. Median and reference values are representative of average behavior rather than extremes. These values cannot be used for highly exposed persons for whom high TEQ will induce higher elimination. However, the proposed method of half-life prediction can be used to relate past and present intake to serum concentrations and is useful to understand the effect of demographic characteristics on serum concentrations.

CHAPTER III

Heterogeneity in Norovirus Shedding Duration Affects Community Risk

3.1 Abstract

Norovirus is a common cause of gastroenteritis in all ages. Typical infections cause viral shedding periods of days to weeks, but some individuals can shed for months or years. Most norovirus risk models do not include these long-shedding individuals, and may therefore underestimate risk. We reviewed the literature for norovirus shedding duration data and stratified these data into two distributions: regular-shedding (mean 14 / 16 days) and long-shedding (mean: 105 / 136 days). These distributions were used to inform a norovirus transmission model that predicts the impact of long-shedders. Our transmission model predicts that this subpopulation increases the outbreak potential (measured by the reproductive number) by 50-80%, the probability of an outbreak by 33%, the severity of transmission (measured by the number of cases) by 20%, and transmission duration by 100%. Characterizing and understanding shedding duration heterogeneity can provide insights into community transmission that can be useful in mitigating norovirus risk.

3.2 Introduction

Norovirus is notorious for causing highly explosive epidemics in semi-closed environments, though it also creates significant disease through endemic community transmission. As the most common cause of epidemic gastroenteritis across all age groups [31], it is responsible for greater than 90% of viral gastroenteritis and about 50% of all cause outbreaks worldwide [92]. In the United States, norovirus infections cause an estimated 71,000 hospitalizations annually, costing nearly $500 million per year [72]. While not as well characterized as its role in outbreaks, its endemic role is major; endemic incidence is estimated at around 5% per year for all ages and 20% per year in children under five [73, 121].

A typical norovirus infection is self-limited, with the shedding period lasting a few days to a few weeks. However, many individuals can shed noroviruses long past symptom resolution [35, 99, 132], and some can shed for months or even years. Asymptomatic shedding has been linked to transmission [90, 120], implying that individuals who shed the virus for long times may have a serious role in sustaining community transmission after an outbreak. Infants are often in this long-shedding group; an immature immune system or the presence of maternal antibodies (found in about 75% of infants <6 months [84]) can lead to extremely long shedding periods. Similarly, immune-compromised individuals, who are unable to fully clear the infection, may shed norovirus for months or even years [34, 46].

Despite these data indicating the presence of long-shedders, and the resultant heterogeneity in shedding duration, current norovirus transmission models assume short and homogeneously distributed durations of viral shedding. For example, of two recent models of nosocomial norovirus transmission, one assumes an infectious

shedding period of less than 2 days [132] and the other a distribution from 1 to 8 days [65], while a recent household transmission model estimates an average infectious period of 1.17 days [142]. These short infectiousness periods may be appropriate in closed environments such as a house or hospital, where symptomatic individuals shed a high magnitude of the virus and quickly exhausts a closely connected and limited population, but they cannot capture the effects of long-shedders in sustaining community transmission.

To better characterize the effect of long-shedding on norovirus risk, we review the existing literature for empiric data on human norovirus shedding duration. These data are used to estimate distributions of shedding duration that include realistic heterogeneity. This information is then used to inform a norovirus transmission model. Using this model, we demonstrate how the presence of a long-shedding group affects risk outcomes including the number of infections, duration of transmission, and probability of outbreaks.

3.3 Methods

3.3.1 Literature Review Search Strategy

We reviewed the literature for individual level human viral shedding duration data through the electronic database Scopus by a keyword search using the terms norovirus paired with *shedding* and/or *excretion*. These articles were supplemented with sources identified from bibliographies of the resultant studies and with unpublished data from known norovirus researchers.

We restricted the results to English language human studies containing individual-level data that were acquired through the use of Polymerase Chain Reaction (PCR). Studies that compare PCR with earlier techniques (electron microscopy and ELISA) demonstrate substantial improvements in detection capabilities [4, 7], and thus more

accurate estimates of viral shedding duration. The main concern with PCR is that it does not distinguish between viable and non-viable viruses, and because noroviruses are nonculturable, there is no way to determine the infectivity of those detected through this method [69]. It is PCR detection data to estimate the duration of infectiousness, based on three points: 1) norovirus studies demonstrate that passage through a human host does not diminish infectivity [123], meaning that shed virues, though not culturable remain viable. 2) Post-symptomatic shedding has been shown to occur in very high concentrations [7, 126, 6], and 3) norovirus has an extremely small minimum infectious dose [141] challenge studies have demonstrated infection with 4.8 RT-PCR units [7], and the estimated average probability of infection for a single norovirus particle is about 0.5 [123]. Even if some viral particles mutated to have non-infectious capsids yet remained intact, only a few would need to remain to cause infection

3.3.2 Literature Data Extraction

In the challenge studies, shedding duration was calculated from the first positive stool sample to the last positive stool sample. For other types of studies (e.g. outbreak investigations), the date of the first positive stool sample was not known, so we assume that viral shedding starts with the onset of symptoms and continues until the last positive sample. If shedding lengths were reported in days after inoculation [7], we subtracted the number of days to symptom onset to account for the incubation period, and for consistency among the data sets. Though pre-symptomatic shedding has been reported in less than 30% of the population [38], it is generally for less than one day [7, 39, 66], and there is no indication that it would affect the different shedding groups differently.

We used two methods to identify the long-shedder population, shown in table

	Regular Shedders	Long Shedders
Operational	>and Competent	Compromised and/or <1
Functional	$\leq$ 34 days	$>$ 34 days

Table 3.1: Criteria for data stratification

3.1. First, we examined the literature to identify individual characteristics potentially related to long-shedding. We stratified the data into a long-shedding group if an individual was identified in the original study as having one or more of these characteristics (*operational long-shedders*). As a comparative method, we defined long-shedders as those whose shedding durations exceeded a set maximum shedding length, regardless of individual characteristics (*functional long-shedders*). We chose the value of 34 days as this cut off, as it was the maximum duration in controlled experiments using only healthy individuals (Moe, previously unpublished data (see B.1, appendix)) [66, 113]).

We fit the data to multiple distributions. Using a likelihood ratio test we found that both the lognormal and gamma distributions fit the data well, but we chose gamma distributions based on biological plausibility [47], precedence [105]], and because this distribution can be included in a deterministic model using multi-compartmental waiting times when the shape parameter is >1 (explained in [142]). The stratified data were fit to gamma distributions using the dgamma function in R [97], which estimates parameters using maximum likelihood.

3.3.3 Model Design

The data from the review were used to parameterize two modified susceptible (S), exposed (E), infectious (I), recovered (R) transmission models (SEIR models) based on the schematic in figure 3.1 and the equations below. We first examine a standard deterministic transmission model that includes a long-shedding group. Second, we examine a stochastic, discrete-time version of the deterministic model.

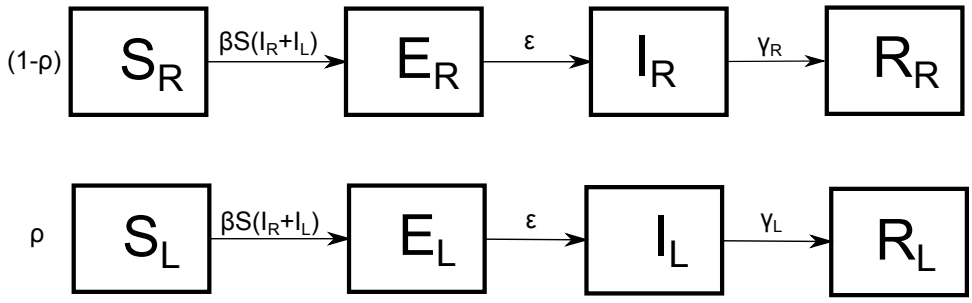


Figure 3.1: Model schematic. S = Susceptible, E = Exposed, I = Infected, R = Recovered, $_R$ = Regular-Shedding, $_L$ = Long-shedding. Parameters: β = transmission probability; $\epsilon = 1/$incubation period; $\gamma_R = 1/$regular-shedding infectious; $\gamma_L = 1/$long-shedder infectious period; $\rho =$ fraction that is long-shedding.

In both of these models, individuals may be in any of the following states, reflecting the within-host stages of norovirus infection: susceptible ($S_{Regular(R)}$ or $S_{Long(L)}$), exposed/incubating (E_R or E_L), infectious/shedding (I_R or I_L), or recovered (R_R or R_L).

3.3.4 Model Equations

$$\frac{\delta S_R}{\delta t} = -\beta S_R(I_L + I_R) \tag{3.1}$$

$$\frac{\delta S_L}{\delta t} = -\beta S_L(I_L + I_R) \tag{3.2}$$

$$\frac{\delta E_R}{\delta t} = \beta S_R(I_L + I_R) - \epsilon E_R \tag{3.3}$$

$$\frac{\delta E_L}{\delta t} = \beta S_L(I_L + I_R) - \epsilon E_L \tag{3.4}$$

$$\frac{\delta I_R}{\delta t} = \epsilon E_R - \gamma_R I_R \tag{3.5}$$

$$\frac{\delta I_L}{\delta t} = \epsilon E_L - \gamma_L I_L \tag{3.6}$$

$$\frac{\delta I_R}{\delta t} = \gamma_R I_R \tag{3.7}$$

$$\frac{\delta I_L}{\delta t} = \gamma_L I_L \tag{3.8}$$

For the deterministic model, vital dynamics such as host births and deaths are excluded because of the relatively short time scale of the simulations. For the same reason, we assume permanent immunity after recovery. We assume the absence of competing strains and co-infection, and that host mixing is proportional. Infectious states (I_R and I_L) are assumed to exert an equal force of norovirus infection due to 1) low infectious dose [123], 2) high levels of asymptomatic excretion [76, 90], and 3) lack of correlation between virus titer in the first positive stool with shedding length [126].

The population is split into two groups: a fixed proportion (ρ) is in the long-shedding group, with the remainder ($1 - \rho$) in the regular-shedding group. When time = 0, an infectious seed of one individual is in the regular exposed state (E_R), and the remainder are in the susceptible states (S_R, S_L). Susceptible individuals become exposed (E_R or E_L) at an average per-infected rate of β, and transition into the corresponding infectious/shedding state (I_R or I_L) at an average rate of ϵ, which is assumed to be the same for both shedding groups. Individuals enter recovered states (R_R and R_L) at rate γ_R for regular-shedders, and γ_L for long-shedders. Estimated parameters used in these simulations are provided in table 3.2.

We assess risk in the deterministic model by examining changes in the basic reproduction number (R_0), which is a measure of the potential of a disease to spread in a population. R_0 is typically defined as the expected number of secondary infections produced by a single index case in a completely susceptible population [5]. It can be expressed as the product of the expected duration of the infectious period and the rate at which secondary infections occur; in a standard SEIR model with a mean infectious period of $1/\gamma$ and a transmission rate of β, R_0 is given by 3.9.

$$R_0 = \frac{\beta}{\gamma} \tag{3.9}$$

In our model, where multiple infectious phases are possible, R_0 is the sum of the expected number of secondary cases generated by an individual in each state, weighted by the probability that the index case will occupy that state. For the model presented here, R_0 is a product of the transmission probability β and the expected time an individual will spend in each of the infectious states I_R and I_L ($1/\gamma_R$ and $1/\gamma_L$) and the probability ρ of being in the long-shedding group (3.10).

$$R_0 = \beta(\frac{(1-\rho)}{\gamma_R} + \frac{\rho}{\gamma_L}) \tag{3.10}$$

We use a discrete-time stochastic model to examine probability, duration, and severity of outbreaks, where individuals can be in the same disease states as presented in the deterministic formulation above (S_R, S_L, E_R, E_L, I_R, I_L, R_R, R_L; Figure 3.1). In this model, we assume a single population with a frequency-dependent contact rate. The model is initialized with a population of size 10,000, with one infected individual at $time = 0$ and the rest of the population in the susceptible states. The total number of new infections Xt is drawn from a binomial distribution (St, δt), where St is composed of both S_R and S_L individuals. The exposed period is assumed to follow an exponential distribution with mean duration $1/\gamma$. The regular-

	Parameter Definition (units)	Mean Value[a] Rate (1/days)	Gamma parameters	Source
β	Transmission rate	0.075		
ϵ	1/length of exposed period	0.76		[132]
γ_R(O)	1/regular infectious period (Operational)	0.061 (1/16.4) 2.2, 7.4	Review	
γ_L(O)	1/long infectious period (Operational)	0.009 (1/105.6)	0.8, 129.1	Review
γ_R(F)	1/regular infectious period (Functional)	0.069 (1/14.5)	2.7, 5.3	Review
γ_L(F)	1/long infectious period (Functional)	0.007(1/136.0)	0.7, 199.4	Review
ρ	Fraction that is long-shedding	0.05		[44, 128]

Table 3.2: Parameter definitions and values used in simulations. [a]indicates value used when parameter was not varied in simulation.

shedding and long-shedding infectious periods ($1/\gamma_R$ and $1/\gamma_L$) are assumed to follow gamma distributions with parameters obtained by maximum likelihood estimation using data collected from our literature review.

3.4 Results

3.4.1 Data Extraction

The literature search resulted in a total of 18 English language studies with individual-level human norovirus molecular shedding duration data, listed in table 3.3 and we received two previously unpublished datasets (for unpublished data see B.1, appendix).

Shedding duration ranged from 2 to 898 days across all studies ($N = 168$ individuals) with total mean and median values of 49 days and 19 days, respectively. Much of the variability in this dataset is explained by stratification of the population on two individual characteristics: compromised immunity and infancy. Sixty-two of the 168 individuals were labeled as immunocompromised and/or infants, thus considered together as *operational long-shedders*, while the remaining individuals were considered

Study	N	Age (years)	Immune Status	Genotype	range(days)	mean (days)	median (days)
Atmar and Estes 2006[a]	16	18 - 50	+	GI.I	8 - 54	28.6	27
Florescu et al. 2008*b*	1	1.8	-	NR	240	240	240
Gallimore et al. 2004b*c*	1	Child	-	rGII.3	156	156	156
Goller et al. 2004a[d]	1	18	+	NR	8	8	8
Goller et al. 2004b[e]	9	79 - 94	+	GII	2 - 20	10.7	10
Kirkwood and Streitberg 2008[f]	9	0 - 2	+(6) -(3)	GII.4,GII.6	2- 100*	30.8	15
Lee et al. 2008[b]	1	0.8	+	GII.2	114	114	114
Leon et al. 2011[a]	13	18 - 48	+	GI.1	8 - 35	21.3	21
Ludwig et al. 2008[g]	9	0 - 16	-	GII.4, GII.3	22-433	95.7	46
Murata et al. 2007[h]	23	0 - 3	+	NR	5 - 47	17.3	16
Nilsson et al. 2003[i]	1	65	-	GII	670	670	670
Parashar et al. 1998[j]	1	Adult	+	GII	10	10	10
Schorn et al. 2010[k]	9	23 - 59	-	GII.4, GII.7	97 - 898	305	230
Siebenga et al. 2008[l]	8	0 - 69	-	GII.4,GIIb-GII.3	35-182	89.3	77
Seitz et al. 2011[a]	9	18 - 50	+	GI.1	4 - 34	25.2	25.5
Simon et al. 2006[m]	12	0 - 24	-	NR	3 - 140	40.4	23
Tu et al. 2008[e]	14	63 - 93	+	GI	8 - 14	25.2	25.5
Westhoff et al. 2009[k]	2	73	-	GII	90 - 165	127.5	127.5

Table 3.3: Description of studies and data used in empirical review (+) = immunocompetent, (−) = immunocompromised, (NR)= not reported, (r) = recombinant, [a]Challenge study [b]Case study of infant transplant recipient [c]Case study of child with genetic disorder [d]Investigation of health care worker [e]Outbreak investigation in an elder care facility [f]Prospective hospital study [g]Retrospective study of paediatric oncology patients [h]Outpatients at a paediatric clinic [i]Case study [j]Outbreak investigation of food handlers [k]Study of transplant recipients [l]2-year hospital survey [m]Outbreak investigation in paediatric oncology and hematology unit

	Operational Regular-Shedders	Operational Long-shedders	Funcitonal Regular-Shedders	Functional Long-Shedders	Total
N (individuals)	106	62	120	48	168
Range (days)	2-54	2-898	2-34	35-898	2-898
Median (days)	13	47.5	13	80	19
Data Mean (days)	16.4	105.6	14.5	136.3	49.3
Standard Deviation	11.7	155.3	8.5	165.0	103.7
Coefficient of Variation	71.3	147.1	58.4	121.1	210.3

Table 3.4: Duration summary from empirical review for operational and functional shedding categories.

operational regular-shedders. Mean shedding lengths between these groups differ by about 90 days (Table 3.4). The immunocompromised group included a child with a genetic disorder [36], transplant recipients [33, 64, 88, 112, 134], pediatric oncology patients [75, 118], and one individual with multiple sustained norovirus infections whose immune status was not evaluated, but was assumed to be immunocompromised [55]. Infants were those individuals whose age was reported as <1 year in the study. It is reasonable to expect differences in shedding length to vary by genotype as well, because of different levels of immune response, but we did not have sufficient data to examine this possibility.

Infants that were immunocompetent shed on average for 22.1 days, 1.3 times longer than those in the regular-shedding group (immunocompetent and >1 year old) who shed for a mean of 16.4 days. Regardless of age, immunocompromised individuals shed on average >6 times longer than regular-shedders (139.1 days for >1 year old and 106.6 days for infants). The observed effect of both compromised immunity and infancy (90.2 days) is less than the expected additive joint effect (128.4 days). We tested for additivity using linear regression in R [97]; the p value for this test was 0.01, prompting us to reject the additive effects model. This indicates that either condition can cause long-shedding, but both together (being both an infant and

immunocompromised) does not result in extremely long-shedding durations beyond the effect of either condition alone.

We estimate the percentage of the United States population in the operational long-shedding group, ρ (infants and/or immunocompromised), to be about 5%. The number of infants born in the U.S. in 2007 was 4,317,119 [44] in a population of 304,059,724 [128], approximately 1.4% of the population. The number of immunocompromised individuals in the United States has been previously estimated to be about 10 million individuals (3.6% of the U.S. population), a number that includes organ transplant recipients, HIV infected individuals, and cancer patients [51].

This operational definition is useful for the *a priori* identification of high-risk individuals and for understanding the causes underlying long-shedding, but was not ideal for our analysis as many cases in our dataset are missing data on age or immune status, and thus cannot be correctly classified using our operational criteria. Further, some individuals who are labeled as immunocompromised, and therefore labeled as long-shedders with our operational definition, actually shed for short durations. We therefore also stratified the data based on actual shedding length (*functional long-shedders* shed for >34 days). The mean of the two groups by the functional definition differ by over 120 days (Table 3.4).

The fitted distribution for the unstratified data is highly variable (Figure 3.2). When we stratify the data based on both of our defined shedding groups (functional and operational), much of this variability remains in the long-shedding group (coefficient of variation = 147.1 operational, 121.1 functional), while the regular-shedding groups are more homogenous (coefficient of variation = 71.3 operational, 58.4 functional) (Table 3.4). Using the law of total variance, the variance of the aggregated populations is 10826.2 for the operational definition and 20989.2 for the

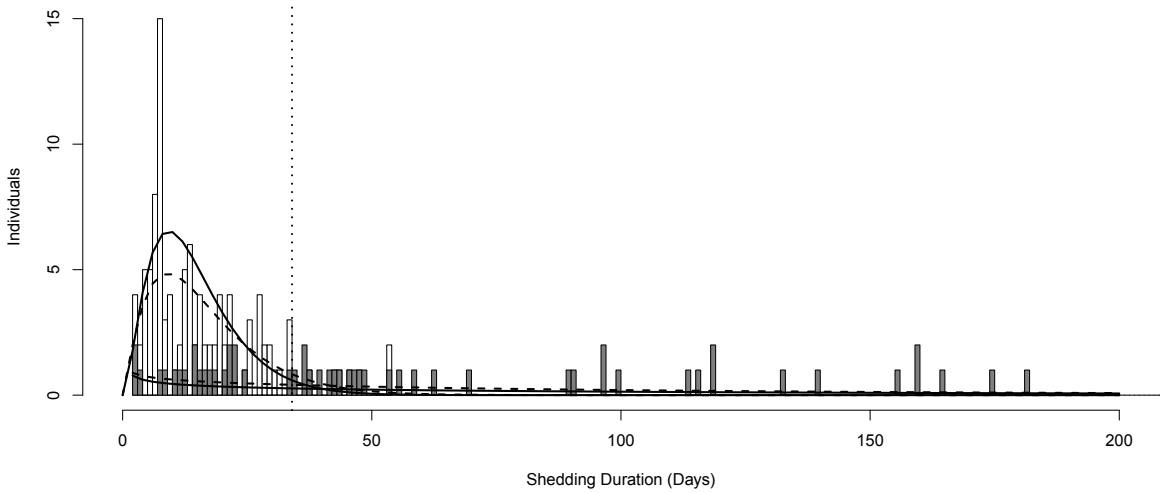


Figure 3.2: Shedding lengths and fitted gamma distributions of individual shedding duration data from empirical review. Data are truncated at 200 days; figure does not include 7 individuals who shed for >200 days (up to 898 days). Gray bars indicate operational long-shedders (infants and immunocompromised individuals) and white bars indicate operational regular-shedders (immunocompetent non-infants). The dotted line at 34 days delineates the cutoff point for the functional definition. Dashed lines represent fitted gamma distributions for the operational definition (Regular-shedder parameters = 2.2, 7.4, Long-shedder parameters = 0.8, 129.1) and solid lines are gamma distributions fitted to the functionally defined populations, where regular-shedders <34 days, long-shedders $\geq$ 34 days (Regular-shedder parameters = 2.7, 5.3, Long-shedder parameters = 0.7, 199.4).

functional definition. By dividing the variance of the means by the total variance, we estimate that stratification into long-shedding and regular-shedding groups explains 35% (functional) to 37% (operational) of the total variance.

3.4.2 Deterministic Model Results

Changes in R_0 determine whether an epidemic will occur as well as the subsequent course of transmission. If $R_0 \leq 1$ transmission will be limited to sporadic secondary infections, and a major outbreak will not occur, whereas if $R_0 > 1$ an outbreak can result. In terms of long-shedding, factors that affect R_0 include the mean duration of shedding of this group ($1/\gamma_L$) and the proportion of the population that is long-shedding (ρ). The effects of changes in these long-shedding related values are independent of the transmission rate, β, meaning that resultant relative increases in R_0 are robust to scenarios of different transmission rates, including environmental or

	$\rho = 0.05$, estimated γ_L	$\rho = 0.05$, estimated $\gamma_L * 2$	$\rho = 0.10$, estimated γ_L
Functional Definition	1.3	1.6	1.5
Operational Definition	1.5	1.8	1.7

Table 3.5: Deterministic model results: Estimated fold increase in R_0 with addition of long-shedders

symptomatic transmission.

Based on equations 3.9 and 3.10, and assuming $\rho = 5\%$, the inclusion of a long-shedding group ($mean$ = 105.6 days (operational) and 136.3 days (functional)), increases risk by increasing R_0 (1.3 fold increase for the operational definition, 1.5 for functional). Because our value of ρ is estimated, we examined the effect of its increase; if ρ grows to 10% of the population (e.g. increased survival rates for those with compromised systems or increase in birth rate), the inclusion of the long-shedding group results in a 1.5 fold (operational) or 1.7 fold increase (functional) in R_0 over a population without long-shedders. Likewise, a two-fold increase in the mean shedding length of the long-shedding group ($1/\gamma_L$) results in a 1.6 fold (operational) or 1.8 fold (functional) increase in the value of R_0 (Table 3.5).

The importance of an increase in R_0 depends on its original value as the number of excess cases that result from an increase in R_0 is highly dependent on β. For example, if R_0 is already quite high (such as within a home), then an outbreak would already be likely to spread through a population and a 2-fold increase would not have much effect. If R_0 is below 1 is or very low, then this same increase can mean the difference of an outbreak dying out or of it taking off in a population(see Figure 1, Koopman and Longini [58] for further explanation). More research needs to be done to determine the community transmission rate to fully understand the effect of long-shedders on R_0.

3.4.3 Stochastic Model Results

Results from stochastic simulations demonstrate that the presence of long-shedders increases the probability that an outbreak will occur while increasing the severity and duration of transmission when disease does not die out. We examined changes in the probability of high transmission events, where high transmission was defined as >200 cases. Based on our simulation results, transmission above this level was sustained long term, and below this level would die out. In the absence of long-shedders ($\rho = 0$, i.e. the entire population draws shedding durations from the functional regular-shedding distribution), there is a 23% chance that high transmission will occur in 1000 runs using our estimated parameters (in Table 3.2). If we allow 5% of the population to draw from the functional long-shedding distribution, the probability of high transmission increases to 31%. This effect is less evident using the operational definition, where the probability of remains about 37% (Table 3.6).

As shown in Figure 3.3, including a long-shedding group by either definition increases both the number of cases, and its duration of sustained transmission. With the addition of long-shedders, the mean number of cases increases 145% (operational) to 225% (functional) over a population with only regular-shedders (Table 3.6). The duration of sustained transmission also increases, approximately doubling for both definitions. Figure 3.3 also demonstrates that these outcomes are dependent on our method of stratification. For example, the number of cases in the absence of long-shedders is much higher using the operational definition (filled circles) than the functional definition (open circles), due to the wider range and higher mean of this subset.

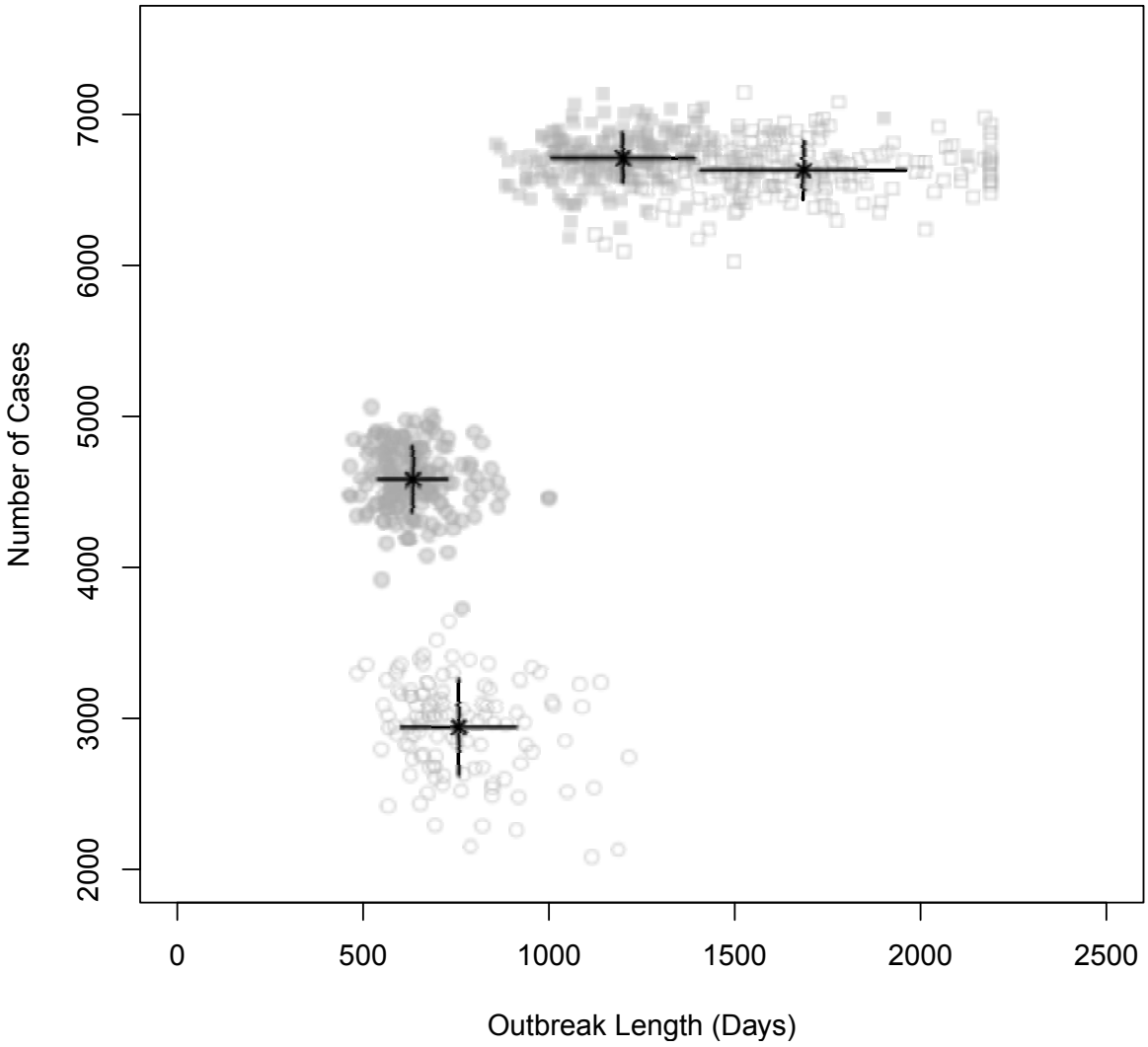


Figure 3.3: Transmission duration and number of cases using shedding length parameter values from our empirical review (3.2). Only model runs resulting in high transmission (>200 cases) are shown. Runs with both long-shedding and regular-shedding groups are represented by squares, runs with only regular-shedders are represented by circles. Filled gray shapes indicate use of the operational (*a priori*) definition and open shapes indicate use of the functional definition (>34 days) for long-shedders. Black lines show the mean and standard distribution for each scenario.

	Probability of High Transmission (%)	Mean cases / Transmission Event	Mean Duration of Transmission (days)
Operational / No long-shedders	36	4584	633
Operational / With long-shedders	38	6710	1199
Functional / No long-shedders	23	2939	756
Functional / With long-shedders	31	6630	1683

Table 3.6: Deterministic model results: Estimated fold increase in R_0 with addition of long-shedders

3.4.4 Sensitivity Analysis

Our estimates are based on an incomplete dataset of all true shedding times ($1/\gamma_L$ and $1/\gamma_R$) due to publication bias and the difficulty of obtaining data on asymptomatic community infection. We therefore examined the sensitivity of our outcomes to over a wide range of values for this parameter. The probability of an high transmission increases by about 10% as the mean long-shedding duration ($1/\gamma_L$) increases from 20 to 250, leveling off at maximum probability of 42% for the operational definition and 34% for the functional definition when γ_L >100 days (Figure 3.4). The higher probability in the operational definition is again attributed to the wider range and higher shedding duration mean of the regular-shedding group, which contains the bulk (95%) of the population.

The total number of infected individuals also increases with $1/\gamma_L$, as shown in Figure 3.5. In simulations in a population of 10,000 individuals, the maximum number of cases is about 8000 for both definitions (80% of the population), and remains well above the average number of cases in the absence of long-shedders (46%, operational; 29%, functional). Transmission duration, measured as the last time at which there is at least one infected individual in the population, increases as $1/\gamma_L$ increases (Figure 3.6). The maximum expected duration of about 2000 days (about 5.5 years) is reached when $1/\gamma_L = 200$ days for both definitions. Again, this is much longer than the average duration in the absences of long-shedders (633 days, operational; 756 days, functional).

3.5 Discussion

Heterogeneity in viral shedding duration has clear implications for norovirus transmission dynamics, and should be included when estimating disease risks. In the case

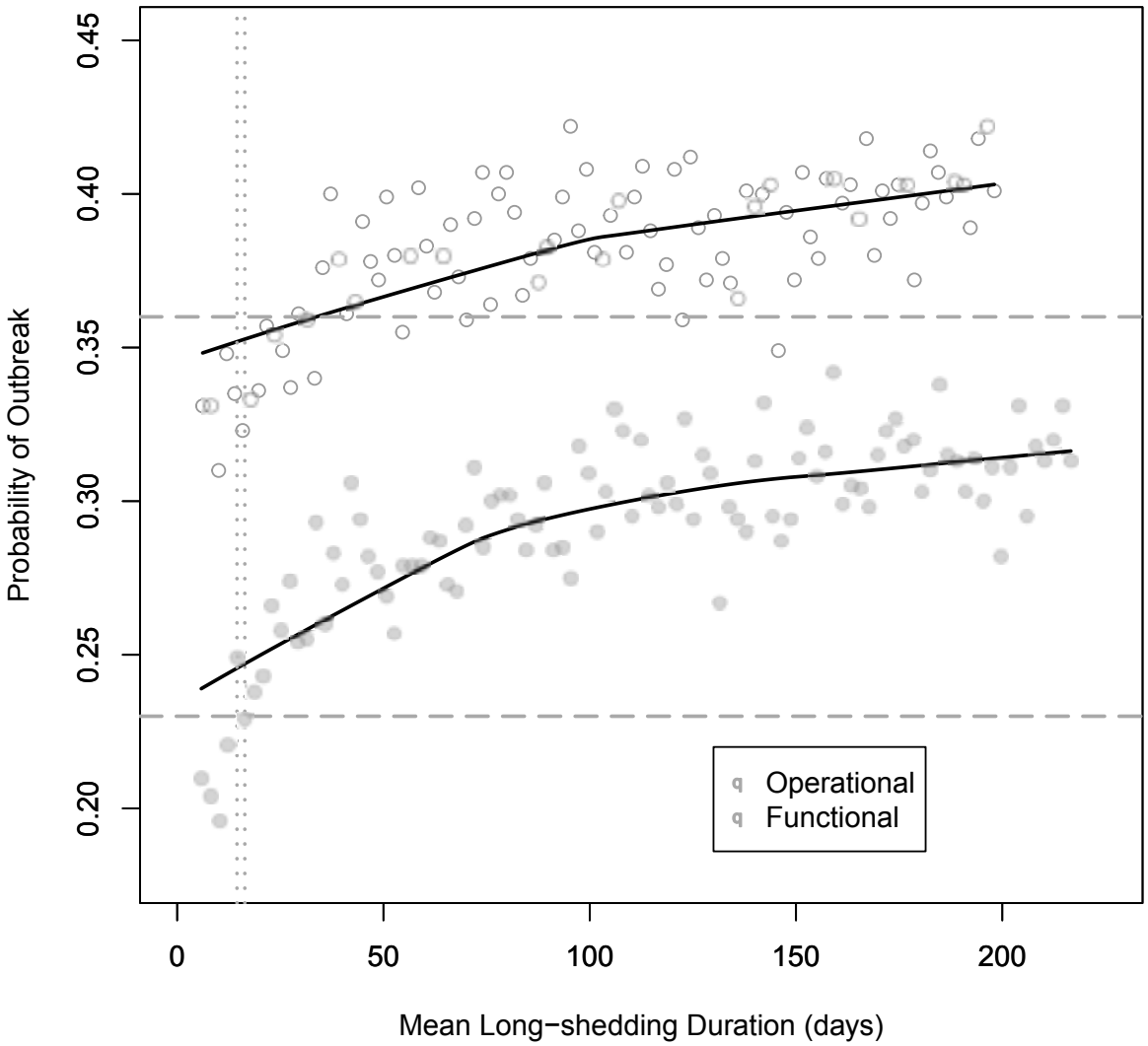


Figure 3.4: Sensitivity of outbreak probability to variable long-shedding duration ($1/\gamma_L$). Outbreaks are defined by having greater than 200 cases. Dashed lines show probability for each definition in the absence of long-shedders, and dotted lines represent the shedding duration of the regular-shedding groups ($\gamma = 16.4$ days, and $\gamma = 14.5$ days). Open circles represent use of the operational definition, and filled gray circles represent use of the functional definition for stratification.

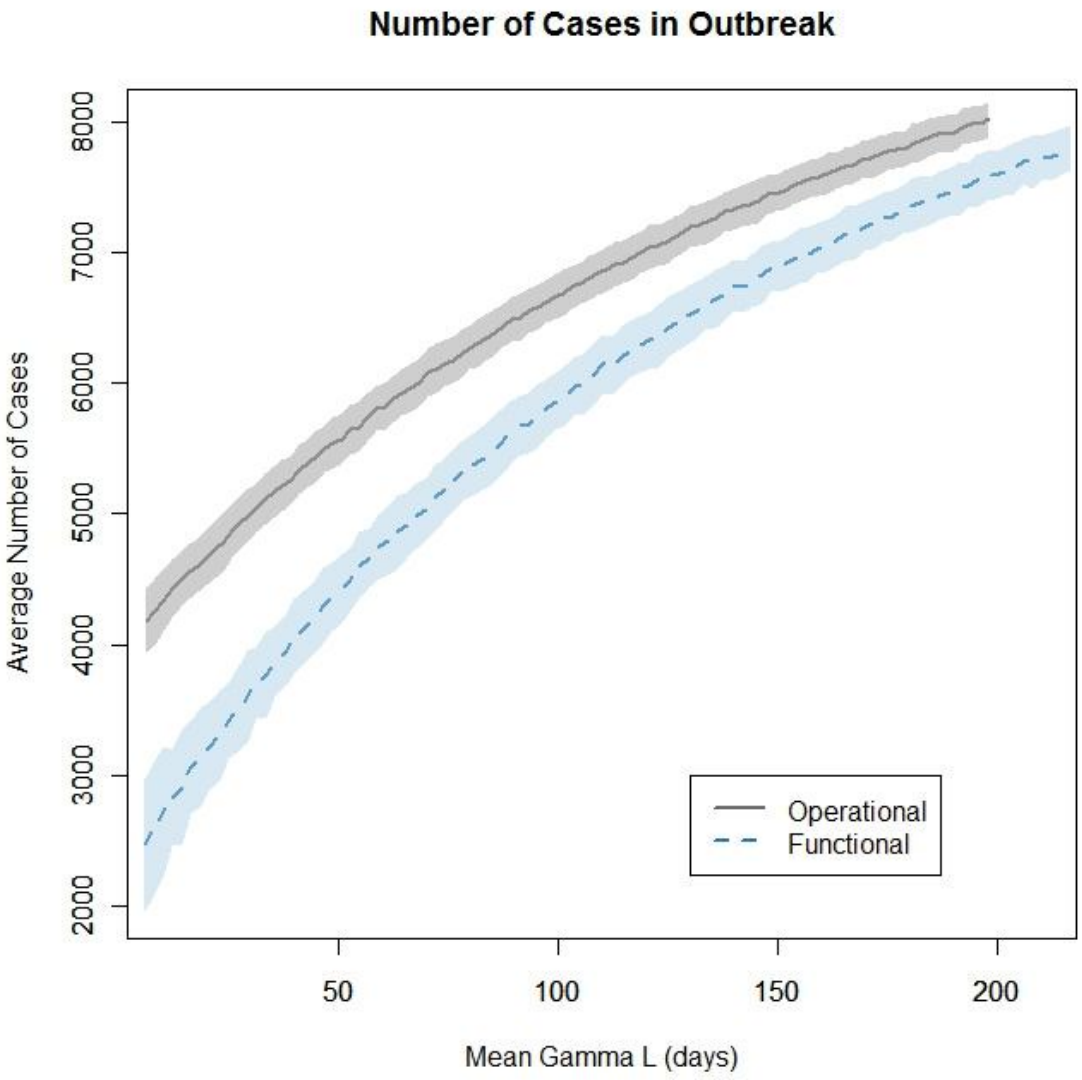


Figure 3.5: Sensitivity of transmission severity to variable long-shedding duration ($1/\gamma_L$). Outbreaks are defined by having greater than 200 cases. Dashed blue line shows average and standard definition for 1000 runs with the functional definition, gray line shows average and standard deviation for operational definition.

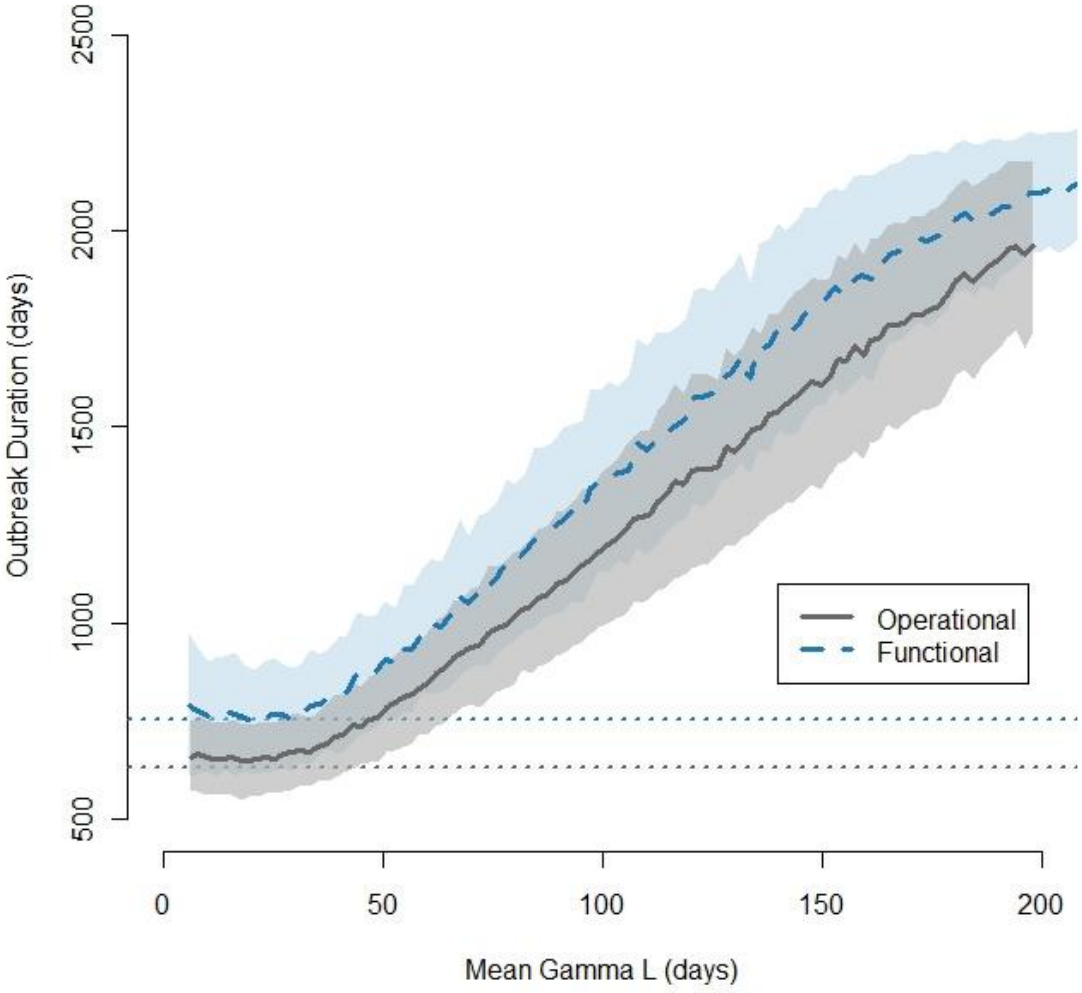


Figure 3.6: Sensitivity of high transmission duration to variable long-shedding duration ($1/\gamma_L$). High transmission is defined by having greater than 200 cases. End of transmission occurs when there are no exposed or infected individuals remaining. Dotted lines show average duration for each definition in the absence of long-shedders.

of norovirus, estimates of short average shedding duration do not capture the potential effects of long-shedding individuals in sustaining community transmission. Our empirically derived characterization of norovirus shedding duration allows us to model its impact on population-level norovirus risk. Our analysis indicates that inclusion of long-shedders results in a 1.5 to 1.8-fold increase in the basic reproduction number R_0, about a 20% increase in the number of cases, and approximately a 2-fold increase in the duration that transmission is sustained in a population.

The simple model in this analysis demonstrates the importance of long-shedders in a single population. It is the first step in understanding the effects of heterogeneity on transmission dynamics, and we expect the results to become more profound with the addition of more complicated and realistic structure. A logical next step would be the inclusion of social structure or multiple populations. In a structured metapopulation comprised of loosely connected groups, a pathogen must persist within its initial group long enough to allow for migration into another one. This type of long-shedder driven persistence may provide an explanation for the explosive but episodic character of norovirus outbreaks: long-shedders may allow the virus to circulate at low levels in the population until it either reaches an individual with a high contact rate (e.g. a food handler), or a pocket of susceptible individuals, (e.g. an unexposed school), resulting in a large new outbreak. A socially structured model could also account for other transmission processes, including clustering of long-shedders in health facilities and other high-risk zones.

The effect of long-shedders may also become more profound if we relax the assumption of homotypic immunity. Immunity has been estimated to last 2-6 months [77], but this is a highly debated topic [27]. When the duration of shedding ($1/\gamma_L$) is greater than the duration of immunity, the long-shedding group can act as a reser-

voir, allowing the infection to persist in a population and potentially re-seed periodic epidemics, in a manner similar to herpes viruses and other pathogens with long-term carriage, such as *Salmonella typhi*.

Because host immune pressure can impact calicivirus RNA evolution [88], long-shedders may also impact the population ecology of noroviruses [41]. Since the amino acids that mutate most frequently are those involved in immune evasion [71, 116], escape mutants may emerge over the life of a single long-shedding infection. In fact, the number of amino acid mutations arising over the course of a year within-host is similar to the number distinguishing outbreak variants from each other [116]. Consequently, long-shedders represent a potential mechanism for introducing novel variants into populations that have achieved herd immunity against the most recent circulating strains.

Our parameter estimates are sensitive to differences in technique and study design across studies (Table 3.3), including differences in fecal viral concentration, specimen storage, RNA extraction efficiency, presence of fecal reverse transcriptase inhibitors, and primer usage [93]. Many studies terminated sample collection while patients were still shedding [7, 39, 88], resulting in right-censored data, and systematic underestimates of actual shedding durations. Publication bias exists in the literature, so that published studies may not reflect the true distribution in the population. Future studies, with increasingly sensitive techniques, will provide more accurate estimates of actual post-infection viral shedding duration.

3.5.1 Conclusions

We have demonstrated the importance of including an empirically validated representation of between-host heterogeneity in norovirus natural history when assessing population-level transmission risks. These results should be incorporated in models

that include other potentially important sources of transmission heterogeneity such heterogeneous contact networks, protective immunity, and strain competition. This will facilitate the development of policies and interventions that can target the individuals who are both the most susceptible and the most likely to transmit disease. As we deepen our understanding of the different types, degrees, and interactions of heterogeneity in disease transmission systems, we can make more informed policy decisions and recommendations and more effectively protect human health.

CHAPTER IV

Travel and the spread of dengue infection in rural Ecuador

4.1 Abstract

Over the last few decades dengue virus has both spread in range and increased in its virulence [61]. Once a milder, urban-centered disease, dengue has moved into peri-urban and rural areas and is now responsible for more serious disease, including dengue shock syndrome and dengue hemorrhagic fever. The factors that put rural areas at risk for dengue virus introduction remain unclear, though travel, urbanization, and lack of vector control programs appear to play a role.

In this work, we examine a rural region of coastal Ecuador undergoing severe environmental change. Blood samples were collected from eight villages with varying levels of accessibility within the region, and were analyzed using a novel ELISA assay. We found that prevalence ranged from about 18% to 57%, with most villages between 25% to 35%. There was heterogeneity in antibody responses both between villages at each time point, and within a particular village over time. We examined potential factors that could lead to this heterogeneity, including age and rates of travel to areas of high dengue transmission, but did not find an association with either of these factors.

We found that there is an increase in travel to areas of high dengue transmission

for villages with road access, but that this increase is not necessarily associated with an increase in levels of infection. This lack of association may be due to confounding through cohort demographics, or because of a threshold effect in travel. The heterogeneity of infection rates through this region indicate that a village-focused intervention strategy may be more efficient at reducing overall infection than one directed at the household or regional level.

4.2 Introduction

Dengue is a class of four flaviviruses that are the cause of the most prevalent arthropod-borne viral disease for humans, causing an estimated 50 - 100 million infections worldwide every year [42]. Over the past 50 years the effect of dengue has worsened; incidence has dramatically increased, and the viruses have evolved rapidly with the more virulent genotypes expanding beyond their historic ranges [61]. The global expansion of its range and incidence is reflected most strikingly in the Americas and Caribbean, where urbanization, insufficient political commitment, increased travel, and inadequate resources have contributed to the re-emergence and increase of dengue in all 19 countries previously certified to be free of its vector, *Aedes aegypti* [122]. Since the 1980s there has been a 4.5-fold increase in the number of reported cases in the Americas alone [104]. In 2008, more than 900,000 cases of dengue and 25,000 cases of dengue hemorrhagic fever (DHF) were reported in this region [91].

In Ecuador, dengue was eliminated during the continent-wide PAHO vector eradication campaign of the 1970s. After the collapse of the campaign, the re-emergence of dengue was first reported in Ecuador in 1988 with a dengue virus (DENV) -1 epidemic, followed by DENV-2 in 1990, and DENV-4 in 1993. Fifteen years after

dengue was first reintroduced, DENV-3 was identified, and all four strains continue to circulate within the country, producing episodic yearly outbreaks and cyclic epidemics.

Dengue is often characterized as an urban disease [43], yet is spreading to rural areas in many countries including Thailand [14, 119], Malaysia [15], India [60, 124], and Vietnam [111]. It is unknown what favors transmission in these settings [24], but one factor emerges as a potential determinant of rural transmission: human travel patterns. Improved modes of transportation allow for migration of viremic subjects from one place to another and may help spread dengue from urban into rural areas [83]. In rural Amazonia, a lack of spatial clustering supported the hypothesis of little autochthonous transmission in rural areas [24], indicating that the infections in rural areas may be caused by repeated introductions from human migration and travel. In fact, it has been shown that dengue is frequently imported into regions with lower transmission through infective sparks from endemic cities [18] and that migration was a predictor of the presence of dengue IgG antibodies at baseline and subsequent seroconversion to dengue [24]. Previous studies indicate a link between road access and increased risk of diarrheal disease [29] and malaria [133]. Along with the increase in travel and migration that comes with road access, dengue vector studies also indicate that mosquitoes are distributed along roadways [28], providing two independent processes that link dengue infection to roadways. The construction of a new road into a rural area in northern coastal Ecuador provides an opportunity to undertake a natural experiment that analyzes the effects differences of road access on dengue transmission.

In this study, we examine differences in dengue seropositivity in a rural region of Ecuador that is undergoing severe environmental change. By measuring levels of

anti-dengue IgG through serological analyzes, we are able to determine the extent of transmission in this rural region and its relationship to road access and local travel patterns. We hypothesize that heterogeneity in infection levels related to variability in road access through variable routes of travel. Understanding the sources of infections and the sources of the heterogeneity the distribution of these infections is crucial for the development of intervention strategies to mitigate dengue infections, to stop its spread into unaffected regions, and to identify those villages most at risk for future outbreaks.

4.3 Methods

4.3.1 Study Area

In the northern Ecuadorian province of Esmeraldas, approximately 125 villages (ranging in size from 20-800 inhabitants) lie along three rivers: the Río Cayapas, the Río Santiago, and the Río Onzolé. These rivers flow toward the town of Borbón (1° 5′ 18" North, 78° 59′ 20" West), a town that serves as the main population center of the region (Figure 4.1). This area is undergoing intense environmental and social change following the construction of a new highway along the coast connecting previously remote villages to the outside world. In 1996, the Ecuadorian government began construction of a road linking the southern Colombian border with the Ecuadorian coast. Construction of the road was completed from Borbón westward to the coast in 1996 and from Borbón eastward to the Andes in 2003. Secondary and tertiary dirt roads off this two-lane asphalt highway are continually being built, mostly for logging and mining, and the area is known as one of the world′s top ten deforestation fronts [85, 117]. In the two cantons where these study villages are located, 55% of the population self-identifies as ethnically Afro-Ecuadorian and 13% as Chachi, the predominant indigenous group [125]; the rest identify as white or mestizo.

Figure 4.1: A. Map of study region, circled area and zoom indicating study region (Google Maps), B. Zoom of study region. Filled circles represent villages with road access, solid circles indicate close river villages (no road access), dashed circles indicate remote river villages.

Unpublished data from the Ecuadorian Ministry of Health indicate that the greatest number of cases between 1998 and 2009 occurred in 2000, with 22,958 reported cases 22. In the province of Esmeraldas, where our study region lies, a peak of 680 dengue fever cases was reported in 2001. From January 2009 May 2010, it recorded 49 cases (average 2.7/month), demonstrating strong seasonality, with incidence peaking January through April [Ministry of Health Esmeraldas Province, personal contact]. Each province is split into smaller zones or Areas. The study region lies in Area 7, which has seen an increase in the number of classic dengue cases over the past few years, but has not yet reported cases of dengue hemmorhagic fever. Dengue transmission is reported year round in this region since 2009, and the peak transmission season does not closely match that of the rest of the province [Ministry of Health Esmeraldas Province, personal contact].

	Road Access	Close, No Road Access	Remote
Highway	Borbón,San Augustín	-	-
Río Onzolé	-	Tangaré	Santo Domingo
Río Santiago	-	Rocafuerte	Playa de Oro
Río Santiago	-	Herradura	San Miguel

Table 4.1: Designation of villages for sample collection

4.3.2 Study Design

A sample of 8 villages was selected *a priori* based on their geographic location relative to Borbón and their level of remoteness (Table 4.1). The village of Borbón and another village along the highway (San Augustín) were selected as having road access. Two villages lacking road access were selected from each of the three river basins: one very remote, and one less remote. Remoteness was determined by estimating the time and cost to travel from the village to Borbón, as detailed in Eisenberg, et. al. (2006) [29]. All households within each village were recruited. In Borbón, we used a previously selected random sample of 1000 househods [29]. Houses were mapped and demographic information was obtained prior to the onset of this study.

4.3.3 Ethics statement

The study was approved by Institutional review board committees at the University of Michigan and the Universidad San Francisco de Quito. Consent was obtained at both the village and household level. All studied households were part of a larger epidemiologic study. Study participants were questioned regarding current febrile illness during the study, and if positive, were seen by a physician.

4.3.4 Data collection

Field staff members visited each household to collect samples of blood after the rainy seasons of 2008, 2009, and 2010. Participation in the study was proposed on a voluntary basis, and verbal consent was obtained at each sampling. A structured

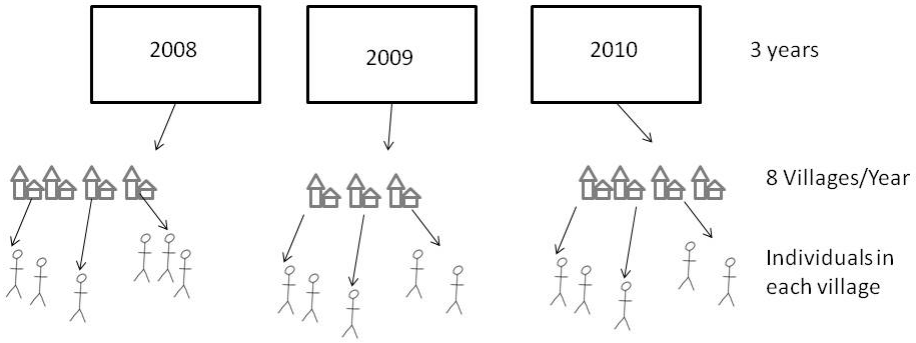


Figure 4.2: Schematic of study design

questionnaire was administered at the house by trained local field staff in Spanish, and approximately 4 drops of blood were collected through finger prick onto filter paper (Whatman 903 Specimen collection paper). Samples were dried, individually wrapped, and shipped at room temperature to the University of Michigan laboratory where they were stored at -80C until analysis.

4.3.5 Laboratory analysis

Blood spots were analyzed for DENV IgG antibodies by means of direct ELISA techniques. IgG antibodies are detectable after the first week of illness, rising dramatically over 2 weeks, after which they level off, but remain at detectible levels [12]. We use natively purified DENV-2 NS5 as antigen to identify the presence of anti-DENV antibodies. DENV NS5 is a non-structural protein that exhibits methyltransferase and RNA-dependent RNA polymerase activities, and is necessary for DENV replication [70]. NS5 proteins have been shown to be immunogenic in induc-

ing antibody responses [115], and NS5 proteins can be used to differentiate between related flaviviruses and flavivirus infection [140], increasing the likelihood of a virus specific response. The protocol for DENV-2 NS5 native purification can be found in the supplementary material.

DENV seropositivity for each blood sample was determined using an IgG direct ELISA, which was performed following standard procedures. Serum solution was attained through eluting two 1/8-inch hole punches of dried blood overnight (4°C) in 350μl 5% nonfat dry milk (NFDM) in PBST (Phosphate buffered saline- Tween 0.5%). Briefly, half of each ninety-six well plate (Nunc-Immuno plates with polysorp coating, Thermo Scientific) was coated with 50μl/well of 135μg/mL purified DENV-2 NS5 antigen in PBS and the other half with PBS only. Plates were incubated overnight (4°C), after which they were washed 5 times with PBS and blocked with 300 μl/well 5% NFDM/PBS-T for 2 hours at 37°C. Plates were washed 2 times in PBS-T and 50μl/well of the eluted blood samples was added in duplicate antigen-positive and control wells and incubated overnight at 4°C. Afterwards, plates were washed 5 times in PBS-T and 50μl/well of the Biotin-conjugated rabbit anti-human IgG secondary antibody (1:20,000 dilution in 5% NFDM / PBS-T; Jackson Immunoresearch) was added for 45 min at 37°C. After washing 10 times in PBS-T, Streptavidin Alkaline Phosphatase (1:2000 dilution in PBST) was added for 30 min at room temperature. pNPP (Sigma Aldrich) substrate system in Tris buffer was used for detection.

The absorbance at OD405 was measured using a BMG labtech FLUOstar Omegaspectrophotometer. Each plate was read at a minimum of 2 time points, fifteen minutes apart: at 15 and/or 30 minutes, 45 minutes, and/or 60 minutes. Each plate contained negative and positive controls in duplicate and each sample was measured in duplicate.

4.3.6 Data Analysis

The duplicate non-NS5 antigen-coated wells were averaged and subtracted from the average absorbance of the NS5 antigen-coated cells for each sample to account for non-specific binding. This final net absorbance value was normalized to the negative sample for that plate to allow for plate to plate comparisons. The final output is therefore reported as fold-increase over negative, called the optical density ratio (OD ratio). Plate to plate variation was minimized by using a positive control from an individual with a documented past dengue infection on each plate. Plates where the positive/negative ratio was more than 3 standard deviations from the mean were re-run. Measurements were included only when the ODE value was above 0.4 and below 2.9 to ensure that the measurement occurred in a region of linearity (not in saturation). Data were double entered and corrected for data entry errors. All statistical analyses were performed in R version 2.12.1 [97].

4.3.7 Cut-off Determination

We used our positive control to establish a cut-off of dengue seropositivity. The mean OD ratio of all of the positive controls was 4.3. As a conservative cut-off, we chose to use 2 standard deviations below this mean, giving us a cut-off value of 2.5. This means that results that were 2.5 times above the negative value (OD ratio >2.5) were considered positive.

4.4 Results

4.4.1 Sample Collection

The study population for all eight villages as determined by the 2009 census included 5587 individuals. In Borbón, the largest village (N=4188), we sampled between 4% - 12.5% of the population. In the smaller villages, sampling coverage

Village	Total N	2008	2009	2010	Unique Individuals	Longitudinal Individuals
Borbón	4118	159 (3.8%)	124 (3.0%)	517 (12.5%)	720	5
Herradura	106	43 (40.6%)	54 (50.9%)	51 (48.11%)	78	24
Playa de Oro	251	149 (59.4%)	84 (33.5%)	203 (80.9%)	237	48
Rocafuerte	141	50 (35.5%)	88 (62.4%)	106 (75.2%)	157	23
San Augustín	333	152 (45.6%)	106 (31.8%)	129 (38.7%)	225	51
San Miguel	143	108 (75.5%)	110 (76.9%)	93 (65.0%)	181	44
Santo Domingo	420	118 (28.1%)	191(45.5%)	240 (57.1%)	345	34
Tangaré	75	22 (29.3%)	33 (44.0%)	34 (45.3%)	63	4
Total	5587	801 (14.3%)	790 (14.1%)	1373 (24.6%)	2005	233

Table 4.2: Number and percent of population sampled in each sample session by village

ranged from 29% - 81% of the village population (Table 4.2).

A total of 2005 unique individuals were sampled in the region. Of the 2964 total samples, about 32% (N = 959) are from the same individuals sampled at least twice during the 3 year period. 233 individuals were sampled for all three sampling sessions, providing a small subset of longitudinal data.

4.4.2 Antibody Levels

The distribution of OD ratios ranges from -1.8 to 40. The majority of all the OD ratios falls between 0.5 and 5.5 (95% quantiles), as shown in figure 4.3 (mean = 2.49, median = 2.10). Negative values are possible when the response in the background wells (no antigen) is higher than the wells with antigen, indicating differences in background only. The very high values shown as the right hand tail in 4.3 may be due to recent secondary infection, as IgG antibodies to NS5 have been shown to rise to high titers after secondary infection [19].

OD ratios are variable both within villages over time and between villages (Figure 4.4). In some villages (Borbón and Playa de Oro), there is a marked increase in

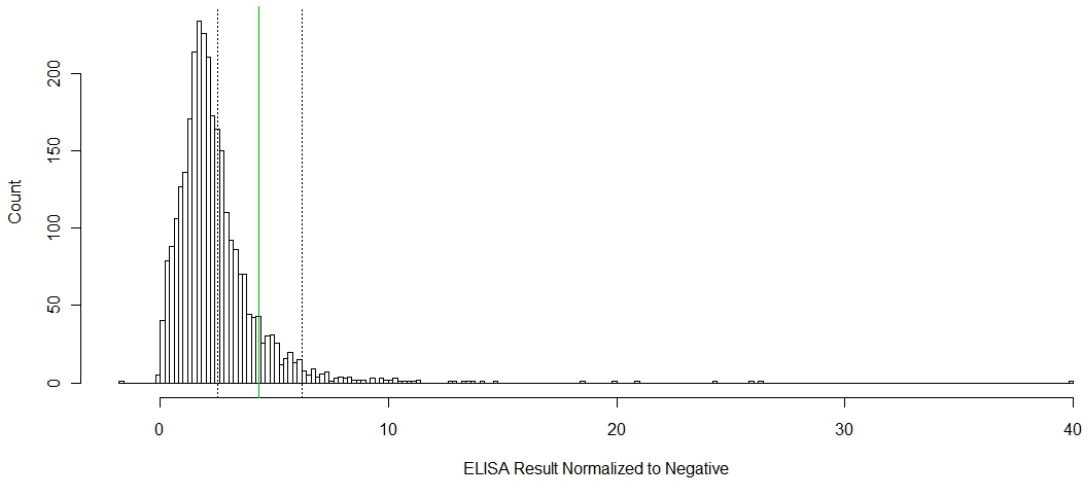


Figure 4.3: Histogram of all ELISA results. Green line indicates the mean value for the positive control for all plates, dotted lines are two standard deviations of this mean. The lower dotted line at x = 2.5 is the cut off for seropositivity (all samples to the right of this line are positive).

Village	2008	2009	2010
Borbón	20.3	36.3	51.5
Herradura	32.6	26.4	37.2
Playa de Oro	25.7	20.2	41.1
Rocafuerte	17.8	56.0	27.3
San Augustín	30.8	20.4	31.4
San Miguel	52.5	29.9	31.5
Santo Domingo	56.8	24.7	35.9
Tangaré	28.6	33.3	29.4

Table 4.3: Percent of individuals seropositive by village and year

mean antibody response over time. This trend is followed, though less drastically, by Herradura and San Augustín. Two villages, San Miguel and Santo Domingo have their highest overall responses in 2008, while one village (Rocafuerte) has the highest result in 2009. In the smallest village (Tangaré, n = 22 - 34), no change is noted. A similar trend is observed for most villages when results are examined in terms of our cut-off. As shown in table 4.3 percent seropositive ranges from 17.8 % to 60% in this region, with the majority of the villages having between 25 to 35

Some of the variability within each village over time may be attributed to a shifting cohort, as only a portion of the population is represented at every time

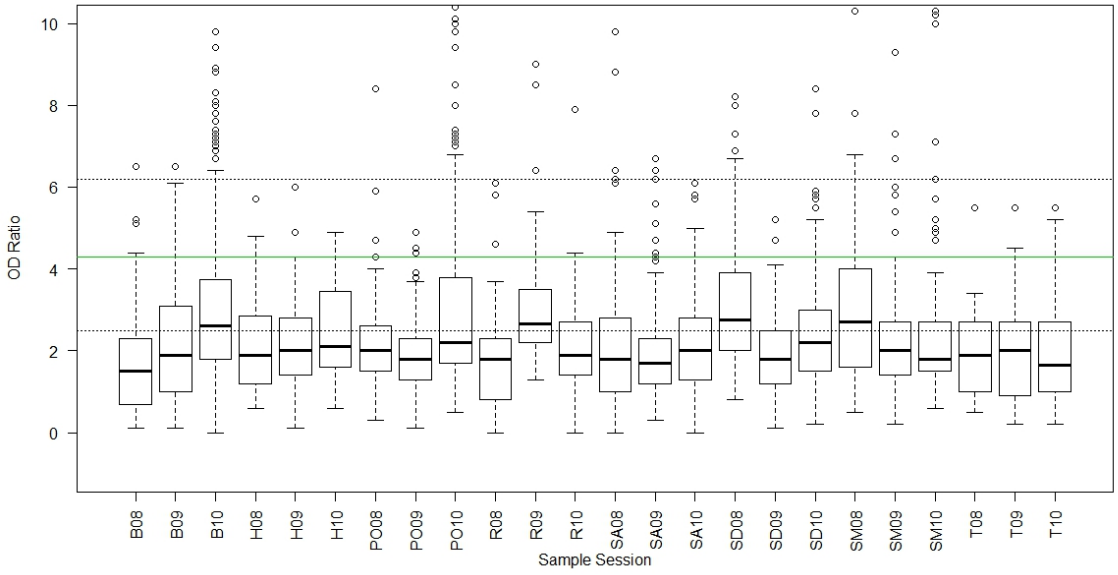


Figure 4.4: Boxplot of sample ELISA results reported normalized to the negative sample (OD ratio). Letters indicate village names and numbers indicate sample year (B=Borbón, H = Herradura, PO = Playa de Oro, R = Rocafuerte, SA = San Augustín, SD = Santo Domingo, SM = San Miguel, T = Tangaré). Figure does not include values <10 times the negative to show variation. The green center line indicates the mean of all the positive samples the dotted lines represent 2 standard deviations from this mean.

point. While we cannot ascertain temporal trends with only three time points, the longitudinal data are useful for understanding within village variation, as shown in figure 4.5. The majority of the individuals in the longitudinal subset did not change serological status (transition from below to above the OD Ratio cut-off of 2.5 or vice versa). In most villages, some individuals transitioned from negative to positive, possibly indicating recent infection. In many of these same villages, however, a similar number of individuals transitioned from positive to negative, indicating a loss of antibody response. This loss could be explained by the drop in antibodies post-infection, or by uncertainty in our assay. There is very little known about the kinetics of NS5 IgG antibodies post infection, and these type of data will become more valuable as this is better understood.

Inter-village differences can be seen through comparison of the cumulative distributions of each village for 2010, the year for which we have the greatest number of samples, as shown in 4.6. The further the curve is shifted to the right, the higher the OD ratios are for that village. For 2010, no clear trend linking road access to OD ratio is visible, as only 2 villages are significantly different at the 5% confidence level, Playa de Oro and Borbón. One of these villages (Borbón) is along the roadway, while Playa de Oro is remote. Statistical differences between the distributions are shown by the Kolmogorov-Smirnov test. The KS-statistics and p values are given in 4.4. In 2010, the cumulative distribution of results for Borbón is different from all villages except for Herradura and Playa de Oro, while Playa de Oro is statistically different from all villages except Borbón, Herradura, and San Miguel.

The two statistically higher villages in Table 4.4 have higher OD ratios for 2010, but this is not true across all time points, as shown in figure 4.4. These incredibly high values during these time points may indicate a recent outbreak, but we do not

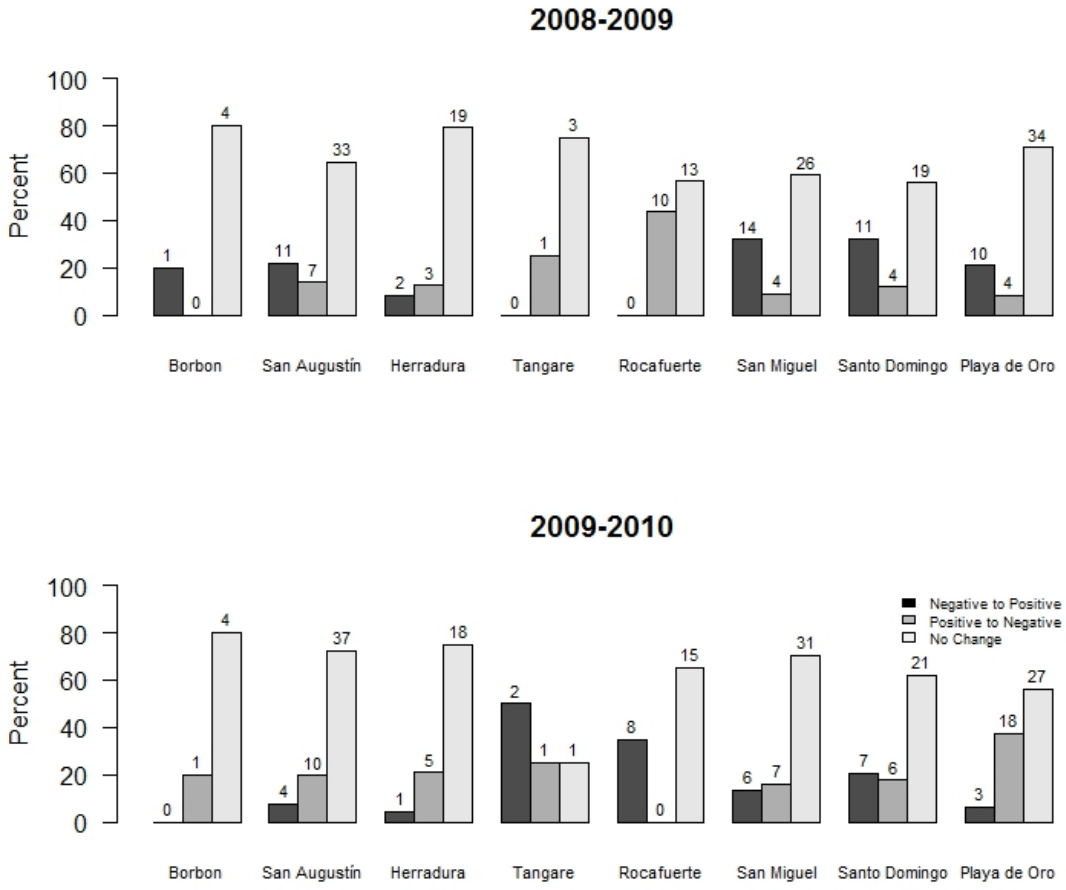


Figure 4.5: Serological transitions for longitudinal samples between timepoints. The top chart represents changes between the 2008 and 2009 sampling period and the lower chart represents changes between the 2009 and 2010 timepoints. Dark bars indicate individuals that were previously negative (OD ratio <2.5) but were positive at the next sampling period, while medium gray bars are the opposite transition. Light gray bars indicate no change in serological status. Numbers at the top of the bars indicate the number of individuals.

	Borbón	Herradura	Playa de Oro	Rocafuerte	San Augustín	Santo Domingo	San Miguel	Tangaré
Borbón	-	0.1632 p=0.1684	0.1309 p=0.0135	0.2271 p=0.0002	0.2002 p=0.0005	0.1493 p=0.0013	0.2436 p=0.0001	0.3248 p=0.0024
Herradura		-	0.1577 p=0.2626	0.1424 p=0.4871	0.1117 p=0.7517	0.0912 p=0.8756	0.1240 p=0.6920	0.2451 p=0.1723
Playa de Oro	*		-	0.2082 p=0.0048	0.1646 p=0.0279	0.1550 p=0.0101	0.1587 p=0.0803	0.2731 p=0.0260
Rocafuerte	*		*	-	0.0711 p=0.9304	0.1112 p=0.3228	0.1088 p=0.6002	0.1432 p=0.6669
San Augustín	*		*		-	0.0688 p=0.8220	0.0873 p=0.8050	0.1512 p=0.5702
Santo Domingo	*		*			-	0.1001 p=0.5123	0.1755 p=0.3182
San Miguel	*						-	0.2030p=0.2562
Tangaré	*		*					-

Table 4.4: KS-statistics and p values for the cumulative distribution functions of the ELISA results for 2010. Asterisks indicate villages with distributions that are significantly different distributions.

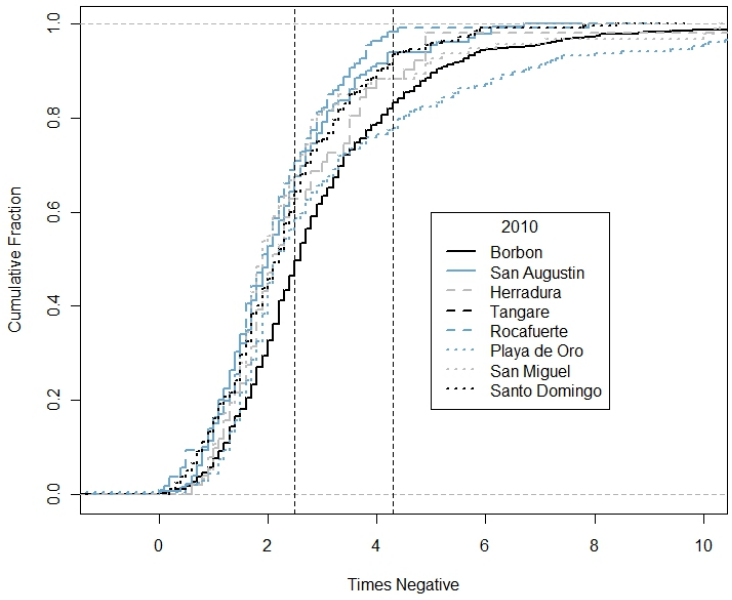


Figure 4.6: Cumulative distribution function for 2010 by village. Solid lines indicate villages with road access, dashed lines indicate those with no road access but close, and dotted lines indicate remote villages. The dotted vertical lines indicate 2 standard deviations below the mean of the OD ratios for all positive controls (x=2.5) and the mean of the positive controls (x=4.3).

have clinical evidence to support this.

4.4.3 Relationship with Age

The differences in OD ratios within villages at different times could be explained by a difference in the ages of individuals sampled for each sampling session. If we sampled the entire population at each time point,and IgG antibodies were persistent, we would expect to see a general upward trend as more individuals become infected. This trend could be masked, however, by an influx of new susceptible individuals, namely unexposed children. There appears to be a similar trend between OD distribution (Figure 4.4) and age distribution (Figure 4.7) for some villages. However, age does not explain the overall increase for an individual OD ratio, as demonstrated in figure 4.8. There appears to be no overall increase in OD ratio with an increase in age, indicating that other factors must also play a role.

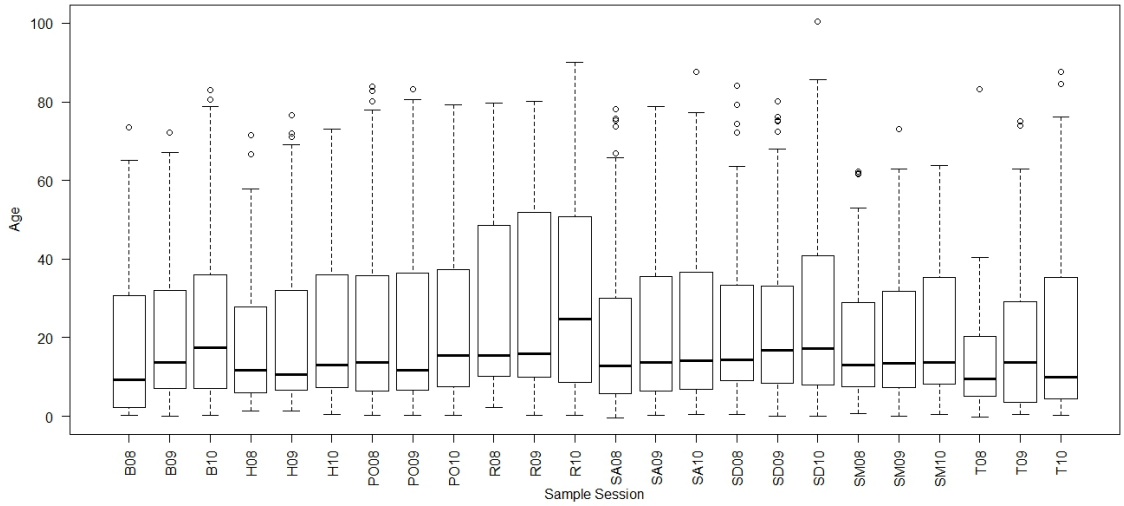


Figure 4.7: Distribution of ages for each sampling session

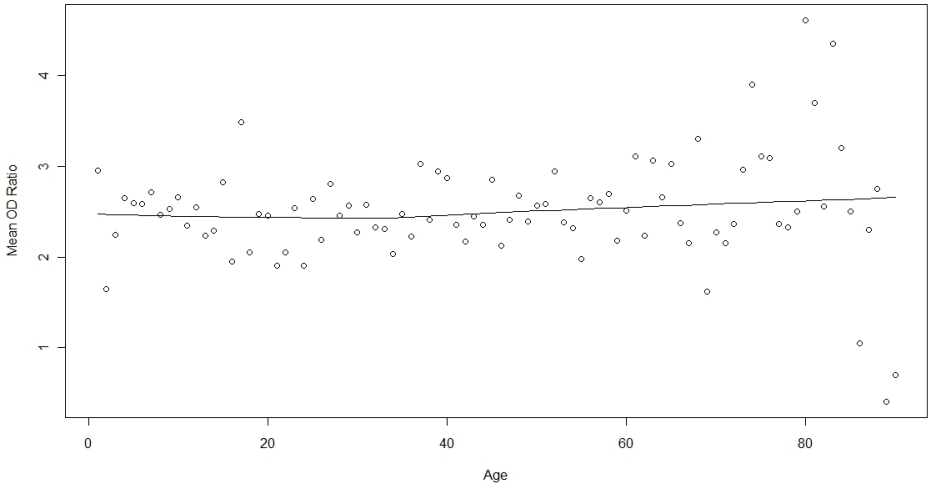
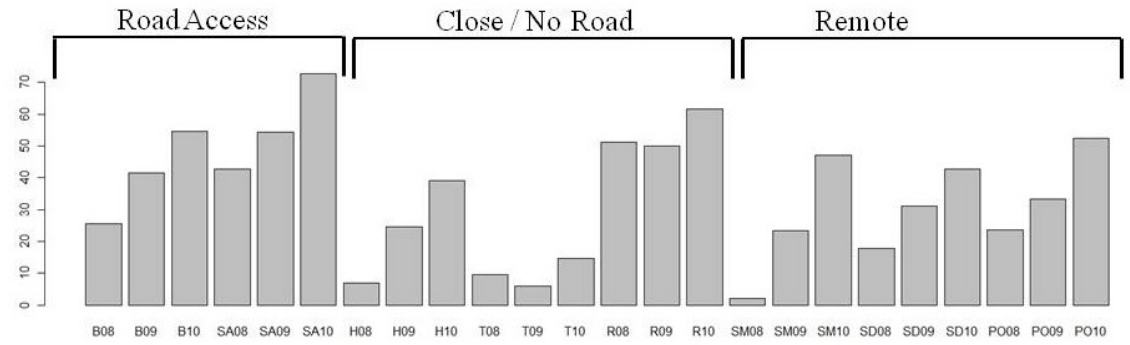
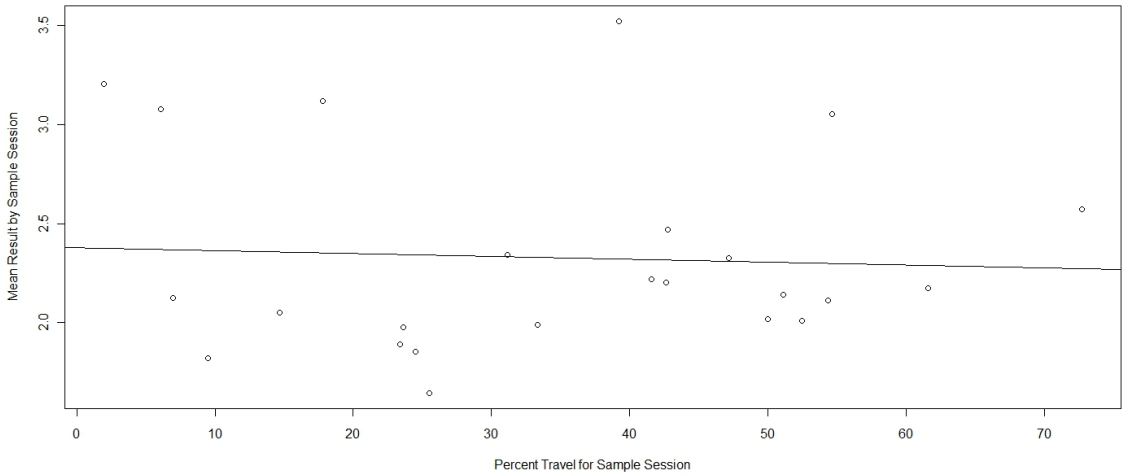


Figure 4.8: Mean OD Ratio by age for entire population



(a) Percentage reporting travel to an area of high known dengue transmission by sampling session and village



(b) Association of travel and OD ratio

Figure 4.9: Travel and dengue over time by village

4.4.4 Relationship with Travel

Travel to areas of high dengue transmission could cause an increase in infection as individuals can both become individually infected while away and can bring back the disease to their villages causing further transmission. During the sampling, we ascertained whether individuals had traveled to areas of known high dengue transmission during the last year. Overall, 1212 respondents (41%) indicated some travel to at least one of these areas. Travel rates ranges from 1.9% (San Miguel 2008) to 72.2% (San Augustín 2010). As shown in figure 4.9(b), we see that travel increases over time for every village, with 2010 being having the highest rate of travel to areas of known transmission.

We also see that villages with road access report generally higher levels of travel than those without access (close and remote). However, no clear association is seen between the mean OD ratio and the percentage travelled 4.9(a).

4.4.5 Discussion

Dengue virus has spread into rural northern Ecuador, infecting many individuals in the area. We believe that its emergence could be linked to a new road that provides access to this region and to areas of known dengue transmission. Our results demonstrate clear heterogeneity between villages at during a particular year, and within villages over our sampling period. This heterogeneity is not fully explained by age differences or rates of travel, indicating a complex picture of transmission in this area. Villages that have road access report higher rates of travel to regions of high transmission, but these rates are not directly correlated with an increase in higher antibody responses. Travel is increasing for the region as a whole, as every year a greater percentage of individuals are travelling to areas of known high dengue

transmission. It is quite possible that we would not see a relationship with this travel, as there may be a threshold above which there is sufficient introduction into the villages to sustain transmission or cause outbreaks.

We anticipated that this relationship would be confounded by age, as older individuals would have a longer time to become infected over their lifetime, and would presumably have higher rates. We do not see such an association, however, as age does not seem to be associated with higher values. This relationship could be demonstrated by following a unique cohort over a longer period of time, and by better understanding the kinetics of the NS5 IgG antibodies over time.

There are technical limitations with the assay that we used in this analysis. There are many advantages in the usage of the ELISA, in that it is cheap, does not require multiple paired specimens, and can be done with blood spots, rather than serum which can be dried and easily transported. However, there is some uncertainty in its response, which can be seen in the wide range of the OD ratio of our positive control, which was used to control for plate to plate variation.

A second limitation is the short time frame of the sample collection. While we can expect a general upward trend as dengue continues to circulate (or a plateau then decrease if transmission is halted), we do not have sufficient time points to capture any temporal trends. Further sample collection and analysis would elucidate any temporal relationships and its continued spread.

We do not see marked differences between villages with road access and those without. This indicates that at this scale, the road does not appear to have an effect. One reason for this could be the great amount of mixing and travel within the region. Our study only examined travel to areas of high dengue incidence, but not travel within the region. Borbón is the major commercial hub of this region, and

acts as a port for many of the goods and services in the area. The road villages may act as a entrance point for the virus to travel up river; individuals from road villages travel to centers of transmission, bringing the disease to the area, and individuals from the various villages can pick up the virus in the major road villages. Finer analysis of inter- and intra- regional travel patterns may make this process clearer.

This type of summary analysis is important in the determination of intervention strategies. If we had seen high levels consistently in the road villages, or across a single river basin, this would indicate usage of a targeted strategy on those areas. Because we see such strong heterogeneity in the region between villages, a region wide approach may not be an appropriate scale for intervention. For example, the high antibody responses reported in Playa de Oro and Borbón in 2010 were not seen in the other villages at that time. In this case, targeted interventions to these villages may be the most cost-effective strategy for this region.

CHAPTER V

Conclusions and Future Directions

5.1 Conclusion

In this work we examine heterogeneity in environmental risk in three very different scenarios. In all cases, we find that heterogeneity can affect quantitative measures of risk. This heterogeneity can arise from many different factors or parameters, and can have varying effects on final risk estimates. Heterogneity may not always affect final risk estimates or health outcomes, and automatic inclusion into risk models may add unecessary complexity. As shown in this work, however, many scenarios require the use of more complex models that include heterogeneity to fully characterize risk and to therefore develop appropriate policy and intervention strategies.

In the first scenario, we show that differences in individual characteristics can lead to differences in excretion rates of persistent pollutants. Excretion rates and half-lives are often modeled as a single value when assessing exposure, but we show that there is a wide range of values reported for many congeners in the literature. Furthermore, individual characteristics such as body fat, age, smoking, and breast feeding affect the elimination of these pollutants. These factors result in heterogeneity in elimination rates between different individuals as well as within an individual over his or her lifetime.

Elimination rates are used to estimate past exposure levels. Many of these chemicals cause a variety of health effects in individuals, including some that arise decades after exposure. By understanding individual elimination rates, and the range of potential half-lives, health professionals can better predict the potential health risks for an individual as well as the potential impact of occupational exposures and industrial accidents. Furthermore, half-life data is necessary for source apportionment, used to determine sources of exposure long after it has occurred by matching particular mixtures in the blood to an emission 'fingerprint'. Because different congeners are eliminated at different rates, the proportion of the congeners at the time of measurement will be different that at the time of exposure. The estimation of the exposure mixture, therefore is highly dependent on the elimination model that is used. Using more accurate half-life estimations will lead to a better understanding of exposure mixtures which can be used to understand past and present exposure pathways.

In the second chapter, we examine how heterogeneity affects risk at a population level, rather than at an individual level. Infectious disease risk is very different than chemical risk in that pathogens can both amplify in the body and can be spread from individual to individual. When we account for differences in the length of time that an individual sheds viral particles, by accounting for the long-shedders in the population, we see increases in the severity and duration of transmission in a community, and an increase in the probability that an outbreak will occur. This heterogeneity in shedding length has not yet been included in models used to develop policies in hospitals or for food handlers, and current policy may not be sufficient to stop transmission in these scenarios. Individuals who are at risk for becoming long shedders are often at greater risk for more severe disease outcomes. The *a priori* identification of these individuals can both aid in their care and allow for

interventions to prevent the further spread of disease.

Norovirus is a complex disease, with a strong environmental component, and many other sources of heterogeneity are likely important, including immunity and social groups. For example, norovirus is thought to cause short term homotypic immunity. If an individual continues to shed the virus for longer than this period of immunity, then the disease can re-enter a population. Heterogeneity in social structure may also have a large effect. Our model of one homogeneous population, while useful for demonstrating the effect of long-shedders, is not realistic. If the population is split into smaller groups with heterogeneous rates of mixing, long-shedders may prove to be key in moving infection between two loosely connected groups. More realistic models that include these and other sources of heterogeneity and their interactions will likely lead to more accurate estimates of population norovirus risk.

Finally, we examine a scenario where population level heterogeneity affects a population level risk. As dengue spreads into rural areas, it is important to understand what factors make a village more at risk for its emergence, including demographic effects, travel, and road access. If we had seen high levels consistently in the road villages, or across a single river basin, this would indicate usage of a targeted strategy on those areas. Because we see such strong heterogeneity in the region between villages, a region wide approach may not be an appropriate scale for intervention. For example, the high antibody responses reported in Playa de Oro and Borbón in 2010 were not seen in the other villages at that time. In this case, targeted interventions to these villages may be the most cost-effective strategy for this region.

Many of the areas that are at risk for dengue are resource limited. As there is no vaccine or treatment for dengue, intervention efforts are focused on vector control. Vector control, while very effective, requires significant resource and community input

and coordination between different villages, government bodies, and organizations. Therefore, it is important to understand the appropriate scale at which to intervene. We show areas of heterogeneous prevalence in transmission in a rural region on a village level. We examine its links to travel and age, but there are many other factors that may be involved as well, including water storage methods, weather patterns, and immunity. As these data are further studied, other sources of important variability may emerge.

Heterogeneity in scenarios of environmental risk can substantially affect outcomes. In this work we examine three very disparate scenarios in which heterogeneity clearly affects risk estimates and health outcomes. As many risk models make assumptions of homogeneity in their estimates, these effects are often missed. By understanding the important underlying variability in these systems, and by using more appropriately complex risk models, we can create more accurate risk estimates that can be used to make more informative policy and intervention decisions for the protection of human health.

APPENDICES

APPENDIX A

Preamble and Chapter II Supporting Materials

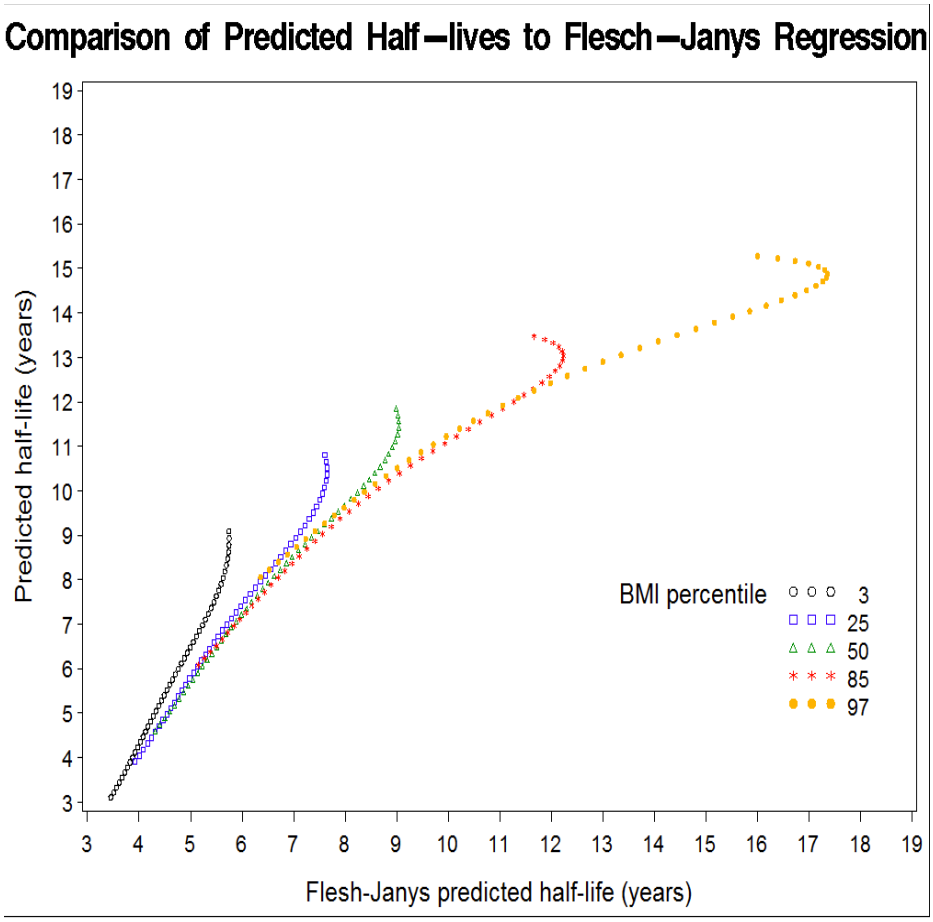


Figure A.1: Comparison of proposed method of half-life prediction to regression presented by Flesch-Janys et a. (1996) for various BMI percentiles

APPENDIX B

Chapter III Supporting Materials

Chapter 3 Supplementary Material

Unpublished Data from Moe CL

Study	Individual	Last Positive sample day post-challenge[a]
a	1	7
a	2	8
a	3	20
a	4	7
a	5	7
a	6	7
a	7	6
a	8	6
a	9	7
a	10	7
a	11	4
a	12	6
a	13	4
a	14	7
a	15	8
a	16	8
a	17	8
a	18	8
a	19	16
a	20	7
a	21	21
a	22	14
b	1	21
b	2	8
b	3	6
b	4	3
b	5	1
b	6	4
b	7	4
b	8	5
b	9	8

Table B.1: Previously unpublished shedding data from two challenge studies conducted by Moe CL. Study a was conducted from July 1994 to December 1998. A total of 75 people were inoculated in these studies (22 became infected). Study b was conducted from October 2000 to March 2002. Samples were collected for a total of 21 days. Study a specimens were examined by RT-PCR using NV-specific primers (NV51/NV3) located in the RNA-dependent RNA polymerase gene as described previously (Lindesmith2003). Specimens from study b were tested by RT-PCR using primers (SR33/SR48) located in the RNA-dependent RNA polymerase gene as described by (Lindesmith2005). [a]Last positive sample day post-challenge defined as the last day stool specimens tested positive for Norovirus RNA as detected by RT-PCR following challenge. For example: if a subject was challenged on 7/1/1995 and their last positive sample was on 7/11/1995 their last positive sample post challenge would be Day 10.

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