Summaries proposals SOR starting in 2009

Title:	**Novel in vitro test for pertussis toxin**
Project number:	**S/360001**
Project leader:	Arnoud Akkermans (VGC – BMT)
Start:	01-06-2009
End:	30-06- 2013
Total costs:	€770000 (2009-2013)

Motivation

Whooping cough (pertussis) is an acute respiratory infection, caused by *Bordetella pertussis.* It manifests as a protracted cough illness. Pertussis toxin (PTx) in its detoxified form (dPT) is an important component of both whole cell and acellular pertussis vaccines (ACVs). Different ACVs comprise different combinations of putative protective antigens of *B. pertussis*, but they all contain dPT as protective antigen. For safety reasons, it is imperative to ensure that the quantity of residual PTx in vaccines does not exceed permissible levels. Therefore, each batch of pertussis vaccine is subjected to extensive safety testing in animals: the histamine sensitization test. However, presence of residual PTx causes major distress, or even death, in the experimental animals. Development of an *in vitro* method is urgently needed.
So far, several *in vitro* assays to detect PTx have been developed, but correlation of the functional *in vitro* test with the *in vivo* tests might remain poor. In the present research project, we propose to develop an alternative *in vitro* method, based on the published knowledge that PTx induces phenotypic changes in different cell types. We hypothesize that the phenotypic changes in cells induced by PTx are preceded by an altered gene expression profile: specific genes are either up- or down regulated. We aim to analyze these differential gene expression profiles using microarray technology. In turn, these marker genes may form the basis of a novel alternative *in vitro* test to quantitatively analyze PTx.

Aim of the project

The aim of the project is to develop an *in vitro* method to detect pertussis toxin in final vaccine formulations. To reach this goal, the following specific objectives of the project need to be achieved:

1. Identification of suitable cell lines, demonstrating phenotypic changes upon PTx exposure
2. Identification of candidate marker genes
3. In-house validation of the *in vitro* method

Strategic and innovative aspects[1]

- The –omics based *in vitro* method will be functional for both isolated PTx and final vaccine products. This proof of functionality is essential for acceptation by regulatory bodies.
- The role of -omics technologies in potency and safety testing of biological medicines, such as vaccines, is almost negligible and research in this area is only in its infancy. The outcome of this research project might enhance the use of -omics technologies in vaccine potency and safety testing for other vaccines besides pertussis vaccines.

[1] . Related projects are: SOR project S/340010 ‘Toxicogenomics in risk assessment’ and EU-project EU project E/360040 ‘Europese Vrijgifte’.

- The –omics based *in vitro* method might serve as a scientifically sound reference method for validation of possible future (cheaper) methods.
- This project will strengthen RIVM's position in future expert advice to regulatory entities.
- The project helps BMT to get in contact with academia in the pharmaceutical field.

Planned activities

The research proposal defines three phases:

Phase 1 (Month 1 – 24): Identification of candidate marker genes in exposed cell lines

We will select a suitable cell line based on a thorough literature study. Besides sensitivity to PTx, suitability for validation versus the classical mouse model will also be taken into account. Selected cell line(s) will be exposed to pertussis toxin per se or final vaccine product spiked with a dose-range of pertussis toxin. Phenotypic changes in metabolism or cell growth will be used to design the test model. Secondly, cells will be exposed to the test "vaccines" in order to generate gene expression profiles using microarray technology. From these expression profiles, candidate marker genes will be identified by performing extensive data analyses.

Phase 2 (Month 25 – 36): Pre-validation of a subset of candidate genes.

Sensitivity and specificity of (a subset of) the candidate marker genes is determined using microarray technology. In this phase we will analyse how far the number of genes to be tested can be reduced without affecting the outcome of the assay. In this phase a choice is foreseen between qPCR and microarray technology.

Phase 3 (Month 37 – 48): In-house validation.

The new *in vitro* method will be subjected to in-house validation. This includes determination of specificity, sensitivity, precision, and robustness. Furthermore, correlation with the classical *in vivo* test method will be determined using available *in vivo* data, derived from on-going batch release testing. BMT has ample experience in validation of methods (ISO17025 accredited).

Planned products

- A high specific alternative *in vitro* method to detect pertussis toxin, preferably in final vaccine formulations, to replace HS testing. HS testing is one of the few animal models in quality control of human vaccines with lethality as endpoint.
- Improved expertise to be used in advice to regulatory entities.
- Endorse the scientific position of RIVM in the EC and other international frameworks.
- At least two scientific papers in peer-reviewed journals.
- Improved opportunities to secure externally-supported projects.

In addition, it will confirm the (international) position of the RIVM (and the Netherlands) as a leading partner in innovative testing and alternatives for animal testing, increasing our access to European grants and consortia. Furthermore it will facilitate future cooperation with academia, as well as ensure cooperation with national laboratories of the OMCL network and vaccine producers.

Foreseen follow-up

If successful, additional inter-laboratory validation in cooperation with interested parties (*e.g.* ECVAM) is to be expected.

Finally, this project can contribute to the EU goal of replacing vaccine (safety) tests using laboratory animals by fully *in vitro* test systems.

Title:	**Population-based Biokinetic Modeling**
Project number:	**S/320001**
Project leader:	Peter Bos (VGC – SIR)
Start:	15-05-2009
End:	15-05- 2011
Total costs:	€303.680 (2009-2011)

Motivation

Humans are continuously exposed to chemicals, some of which are beneficial (e.g. nutrients) while others may induce health risks (e.g. contaminants in food). In assessing the health implications of such exposures one often has to rely on data that do not directly relate to the target population and/or to the exposure situation considered. For instance, although many risk assessments concern peak exposures the impact of the height and frequency of peak concentrations on target tissue concentrations and thus on the occurrence of health effects, is largely unknown. Another relevant policy-driven question is how variability in physiological parameters (such as body weight, age, gender, or ethnicity) or temporal changes in population composition (e.g. ageing of the population or ethnic differences) or in physiological characteristics (such as obesity) have to be accounted for in health assessments. The usual approach of dealing with uncertainties is to apply assessment factors which are assumed to be conservative, resulting in human intake values that should be safe. However, in many risk management problems a conservative approach does not suffice. In addition, it is unclear if the default assessment factors are indeed conservative: they might not be sufficiently protective in some cases, while being overly conservative in others. Similar considerations play an important role when considering the health benefits of nutrients or food supplements.

Aim of the project

The objective of the present project is the development of a generic PBPK-model (=Physiologically-Based Pharmacokinetic model) that will provide insight in how the biokinetics of chemicals depend on chemical-specific characteristics and on biological variability in physiological parameters and that can be used to answer all sorts of generic questions. As a knowledge institute with expertise in (functional) foods, hazard and risk assessment including chemical exposure assessment and (PBPK-)modelling, RIVM has the potential to combine these expertises and develop such a generic PBPK-modelling tool. This model will be adaptable to specific classes of chemicals and will be sufficiently flexible to address a wide variety of chemical- and/or exposure specific situations.

Specific objectives of the project

Objective 1a: Database describing (healthy) human subpopulations of interest in terms of physiological and biokinetic parameters (for instance age, BMI or ethnicity).
Objective 1b: Database describing specific groups or classes of chemicals based on relevant physico-chemical characteristics determining their biokinetics.
Objective 2: A generic PBPK-model to predict the internal dose in (healthy) human (sub)populations following exposure to different classes of chemicals and to compare the associated potential health impact in different subpopulations.

Strategic and innovative aspects

PBPK-modelling has successfully been applied to several high-priority chemicals. However, this type of modelling is data-demanding, and its use is limited to that chemical only. In order to substantiate the support of the majority of policy driven questions, a less detailed and more generic approach is required to address two problems:

1. Repeated/intermittent exposures to peak concentrations.

2. The question what the effect of changes in population. The PBPK-model will be constructed such that once this tool is developed other important topics in health assessments may be addressed as well.

The PBPK-model will be constructed such that once this tool is developed other important topics in health assessments may be addressed as well. Depending on the progress these topics may be incorporated in the present project or in future projects.

Planned activities

Activities during the two-year project include the following steps:

1. A literature search and international research programs will be screened to identify relevant databases and tools to model inter-individual anthropometric and relevant PBPK-models that have a more generic character.
2. Classes of chemicals will be defined predominantly based on various properties (e.g. physicochemical characteristics, slowly or rapidly metabolized) that are important for the fate of a chemical in the human body (absorption, distribution, elimination).
3. A 'standard' PBPK-model will be built containing both a rat and a human model. It will be examined which parameters are specific and crucial for these questions.
4. In the generic PBPK model, the values of the parameters are defined for a specific class of chemicals and/or for a specific human subpopulation in terms of ranges of parameters in the PBPK model. In this way, a series of class-specific PBPK-models is defined. Then, these models are used to study the relationship between external and internal dose. A performance assessment will be made.
5. The next step is to further develop the model along two lines. The first line concerns the question what the effect of changes in population composition may have on the health effects induced by a chemical substance and to subsequently quantify these relationships. The second problem concerns exposure to peak concentrations or intakes for which at present no satisfactory risk assessment methodology is available.
6. Organization of a workshop with stakeholders.

Planned products

1. Database on distributions of physiological and biokinetic parameters for human (sub)populations.
2. Database on various parameters relevant for PBPK-modelling for specific chemical classes.
3. Generic PBPK-model that can address a number of generic policy driven questions.
4. Workshop with stakeholders at the end of the project.
5. Knowledge on quantitative relationships between chemical properties, physiological/biological parameters and health effects that will improve health impact assessments.
6. Reports on description of the databases on distributions of physiological and biokinetic parameters and on various parameters relevant for PBPK-modelling for specific chemical classes and on the performance assessment of the generic PBPK-model (go-no-go decision).
7. Publications on the description of the generic model, including the performance assessment; on the health assessment of specific exposure scenarios by the generic model; on the health assessment for different subpopulations by the generic model.

Foreseen follow-up

The database will be valuable for future health assessments, either with or without modelling, and can be used for new assignments. The generic model will be used to answer policy driven questions in the future and will be supportive for health risk and health benefit assessments, especially for the ministries involved in these fields.

Title:	**Timeliness of response during outbreaks**
Project number:	**S/210076**
Project leader:	Mirjam Kretzschmar (CIb – EPI)
Start:	01-09-2009
End:	31-08-2013
Total costs:	€322.500 (2009-2013)

Motivation

In view of the threat of a future pandemic outbreak of any highly pathogenic pathogen strain, including influenza, with possibly devastating numbers of deaths, health authorities all over the world are designing plans to prepare adequate responses to such an outbreak. Possible intervention strategies for pandemic influenza range from treatment with antiviral drugs, contact tracing and isolation, increasing social distances by closing schools and other public meeting places, to vaccination. The use of mathematical and simulation models has become an accepted means to test and evaluate the effectiveness of different interventions.
The effectiveness of a response in containing an outbreak is largely determined by three quantities, firstly the completeness, secondly the timeliness and speed of every link in the response chain, and thirdly the effectiveness and coverage of reaching individuals who are targeted by intervention. Completeness is determined largely by the awareness of reporting GP's. Timeliness and choice of interventions are determined by the clinical course of infection and its transmission dynamics and by the diagnostic tools available to (rapidly) identify infected individuals. The problem of underreporting of infectious diseases may serve as an example for the interaction of infection, response chain and control measures.
Another point of attention is the behavior of populations. Understanding and making use of the influence of social networks and the underlying mechanisms of decision in conflicts between individual and population interests will greatly enhance the ability of rapid response mechanism to adequately roll out public health interventions.

Aim of the project

The aim of this project is to develop a theoretical framework in which to (a) classify (newly emerging) pathogens according to properties that determine in which way they challenge the public health response; (b) identify the steps of the response chain that constitute an adequate response to any outbreak; (c) identify the extent of underreporting for notifiable diseases; (d) develop a mathematical model that can simulate and analyse the interaction between pathogens and a generic outbreak intervention; (e) use the model to identify the crucial and possibly weak links in the response chain, quantify their expected duration based on empirical data, and quantify the effectiveness of interventions; (f) investigate how the properties of the interaction between outbreak of a pathogen and public health response might change in times of crisis; (g) formulate recommendations for a flexible and regionally oriented intervention strategy; (h) evaluate how changes in reporting responsibilities as defined by the new law on Public Health might affect timeliness and reporting; (i) identify region specific key components for improvement of the reporting to respond chain.

Strategic and innovative aspects[2]

The first innovative aspect is the idea to dissect the transmission and response process into smaller interacting units and to look at the relationship between infectious agent and response in a generic way by viewing them as one interacting system. The idea to investigate the relationship between social network aspects of human behaviour and the implications for the effectiveness of outbreak response implies a combination of methods from social sciences and mathematics/statistics to come to new insights for public health policy. Investigating

[2] Related projects: the project is related to the ongoing SOR project Tracking emerging epidemics.

relationships on the regional level will give new insights into the relationship between the demographic and ethnic characteristics of a population and the effectiveness of outbreak response. Finally, we plan to use concepts from game theory and game theoretical aspects of social network interactions, to develop a flexible way of thinking about response planning.

Planned activities

1. Classification based on literature review of known (emerging) infections according to their clinical and transmission features determining how an outbreak progresses and where response measures can intervene.
2. Identification of relevant response measures and description of the response chain.
3. Identification of crucial factors to quantify the reporting delay. Similarly, identification and measurement of factors determining patient delays.
4. Collection of empirical data concerning (a) clinical and transmission features identified in 1 for some selected (emerging) infectious diseases and (b) quantification of duration and effectiveness of steps in the response chain as identified in 2 and 3.
5. Collecting empirical data concerning regional differences in the response chain for (emerging) infectious diseases through the GGDs in the Netherlands
6. Description of steps in the response chain that are influenced by social networks of target population
7. Development of mathematical framework to describe interaction of outbreak and response chain
8. Calculation of size and extent of underreporting for a set of specific diseases, and modelling the effect of underreporting in timeliness of outbreak detection
9. Incorporation of social network effects into model
10. Investigation of game theoretical approach to effectiveness of intervention measures
11. Identification of weak links in the response chain, most effective intervention strategies depending on type of (emerging) infectious diseases, data needs for better evidence base of intervention
12. Formulating recommendations for improving effectiveness of the response chain and for formulating public health messages (social network and behavioural effects).

Planned products

The main product of the project will be a PhD Thesis consisting of at least 5 papers published or publishable in international journals and two papers on size and effect of underreporting. The papers for the PhD thesis could focus on: (a) a classification of infectious diseases in terms of time scales of relevant dynamic processes and interventions; (b) model definition and analysis of some example diseases; (c) case studies for specific infectious diseases based on notification data (e.g. new influenza A|H1N1 notifications); (d) analysis of the possible impact of contact tracing for specific infectious diseases (e) regional differences in the effectiveness of response. Further products:

- A mathematical model, to collect the relevant data for parametrizing the model and to properly interpret the modelling results,
- A tool for improving completeness and timelines of the reporting and response chain.
- Recommendations for regional improvements in the reporting and response chain.
- Measurements of the effect of implementation of the improvements in the reporting and response chain in different GGD regions.

Foreseen follow-up

The results will contribute to identify those links in the response chain that are most amenable for improvement in practice. Furthermore, it aims at a generalization of the contingency planning as is now conducted for pandemic influenza and can potentially help preparing the public health system for outbreaks of yet unknown pathogens. Finally, the results will be used

to formulate region specific recommendations for improving the performance of the local response to infectious disease outbreaks.

Title:	**Who infected whom**
Project number:	**S/210066**
Project leader:	Jacco Wallinga
Start:	01-01-2009
End:	31-12-2012
Total costs:	€489.200 (2009-2012)

Motivation

Key questions in infectious disease epidemiology are: "how effective are implemented control measures in reducing transmission of infection?" and "how effective should control measures be to control an epidemic?" Both questions can be answered if we have information on two key variables that describe spread of infection: the generation interval τ and the effective reproduction number R. We can estimate these two key variables if we know exactly who infected whom. The real problem in answering questions in infectious disease epidemiology, therefore, comes down to answering the question "who infected whom?"

Infectious disease epidemiologists collect data on the epidemiological characteristics of infected cases, such as their age, gender, time of symptom onset. This provides a valuable source of information that allows us to make epidemic curves. Microbiologists and virologists, on the other hand, are characterizing the fingerprint of pathogens using molecular techniques, for example using the sequence of nucleotide pairs of viral RNA. This provides a valuable source of information that allows us to make phylogenetic trees. The 'traditional' epidemiological data allows for inference of the effective reproduction number R and the generation interval τ. Also the 'molecular' sequence data allows for such inference. As methods for molecular sequencing become faster and cheaper, it is increasingly common to have both reliable 'epidemiological' data and reliable 'molecular' sequence data. The foot-and-mouth outbreak in the UK in 2001 and the SARS epidemic in Singapore in are perhaps the best known examples of infectious disease outbreaks with both 'traditional epidemic' and 'molecular sequence' data. For both the SARS and foot-and-mouth outbreaks the estimated transmission paths resulting from molecular sequence data did not always match up with those of traditional epidemiologic data. This begs the question how we can combine both sources of information to come up with a single and improved integrated analysis of transmission paths and parameters to underpin our recommendations for infectious disease control.

There exists a range of methods that bridge the gap between analysis tools for 'molecular' sequence data (phylogenetic trees) and analysis tools for 'traditional' epidemic data (epidemic curves). The availability of this range of methods would allow us to tailor analysis tools to the data at hand. Rather than throwing data away because it cannot be used in the analysis, we can use all the epidemiological and molecular sequence data we have to reconstruct who-infected-whom in a single analysis, estimate the key epidemiological parameters, assess effectiveness of control measures, and use this to underpin decisions for infectious disease prevention and control.

Aim of the project

Our extremely ambitious aim is to integrate traditional epidemic data and molecular sequence data, and reconstruct transmission paths and ancestral lines of the observed pathogen sequences, as well as key epidemic parameters such as the reproduction number and generation interval that are necessary to assess the effectiveness of interventions. That is, given the observed case reports and molecular sequence data, we aim to answer the question "who infected whom?"

Strategic and innovative aspects[3]

This project bridges a gap between analysis tools for molecular sequence data and analysis tools for traditional epidemiological data. A separate analysis is likely to result in two contradictory conclusions from two different sources of information. We must have methods that use one single analysis of all available data to underpin decisions about infection control.

Planned activities

1. We will rephrase coalescent theory in the terminology that is used for describing infectious disease transmission models.
2. Overview of available data: this is key to measuring whether the new methodological lead to advancement of our understanding of infection transmission, and to improved precision of our estimates of epidemic parameters, and to correct assessment of the effectiveness of interventions.
3. Next we will derive a joint distribution for generation interval and number of nucleotide substitutions between two pathogen sequences that are sampled from a secondary case and its primary case.
4. (a) To further extend earlier work we will first adapt existing methods to analyze epidemiologic data to allow for missing or misclassied links between cases. (b) Reconstructing links to unobserved (asymptomatic, unreported) cases in incompletely reported disease surveillance: Tuberculosis as an example. (c) By reconstructing links to observed cases (4a) and links to unobserved cases (4b) we have all ingredients to build a transmission tree.
5. Inferring key epidemiological parameters, including effectiveness of interventions, from reconstructed transmission trees.

Planned products

Publications in international peer-reviewed scientific journals :

1. Coalescent theory for infectious disease epidemiologists: a tutorial.
2. Frequency of observed differences between pairs of infector and infectee: towards a joint likelihood function of the generation interval and the genetic distance.
3. Reconstructing missing links in completely reported infectious disease outbreaks: pneumonic plague as an example.
4. Reconstructing properties of asymptomatic, unreported and missing cases in incompletely reported disease surveillance: hepatitis B as an example.
5. Reconstructing transmission trees using likelihood-based methods and pair-based likelihood functions.
6. Inferring key epidemiological parameters, including effectiveness of interventions, from reconstructed transmission trees.
7. A PhD thesis.

Foreseen follow-up

Direct applications of the tools developed in the proposed project could include the following:

- Making a scientifically sound statement of the probability that one case has infected another when such statements are required in court;
- Providing clear directions for which type of contacts to include and exclude in epidemiological contact tracing for control of tuberculosis;
- Assessing the impact of an intervention during an outbreak, using all collected epidemiological and molecular sequence data, even when a vast majority of cases is asymptomatic, even if few cases are sampled to obtain the molecular sequence of the

[3] Related projects: This project builds heavily on the expertise accumulated in the ongoing project, S/210046 "Epidemic Modeling of Molecular Data".

pathogen, and even if the various pathogen strains have evolved different epidemic characteristics.