

## **Research Exam Q7, biomedical sciences – 2016-2017 April 5, 2017**

During the exam you have access on a computer to these books:

**Casarett & Doull's Essentials of Toxicology (3e);**

You are allowed to use a calculator of the type Casio FX-82MS.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

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## Screening

### Question 1 - Colorectal cancer screening (17 pt)

Colorectal cancer is an important health burden. In the Netherlands colorectal cancer was diagnosed in more than 15.000 individuals (2015) and about 5.000 people died from this disease (2014). In 2012 the 5-year survival was between 62 and 65%. The rationale behind colorectal cancer screening is that a cancer develops from adenomatous polyps. These polyps, especially when bleeding microscopically, are fairly easy to find and remove. There are various tests available that could be considered for use as a screening test, see table below (based on table 4 from the Dutch Health Council Report on colorectal cancer screening, published November 2009).

*Table - Expected outcomes for five screening tests in one screening round. Percentages.*

Screening test	Sensitivity for advanced adenomas	Sensitivity for colorectal cancer	PPV for advanced adenomas	PPV for colorectal cancer	Risk of complications without further assessment (colonoscopy)	Risk of complications including colonoscopy	Participation
<b>Colonoscopy</b>	>90	97	6,7	0,8	0,1	not applicable	20-25?
<b>CTcolonography</b>	>90	97	40-67, depending on cut-off	5-9, depending on cut-off	very small (probably <0,00005)	0,02	35?
<b>gFOBT</b>	12	20	41	10	0	0,006	47
<b>iFOBT</b>	27	65	40	8	0	0,017	60
<b>Sigmoidoscopy</b>	55	60	79	6	0,002	0,026	30

In 2011 the Minister of Health, Welfare and Sports decided to implement a national screening programme for colorectal cancer starting in 2014. The target group consists of men and women aged 55 to 75 years (about 4 million). This group will receive a biennial invitation to provide a stool sample that will be checked for traces of blood using the immunohistochemical Faecal Occult Blood Test (iFOBT). Traces of blood can be caused by colorectal cancer or polyps, but can also have other causes. The expected participation in screening at that time for iFOBT was 60%. Applying a cut-off value of 75 ng/ml it was expected that 6,4% of the screened persons would have a positive iFOBT. The sensitivity and specificity for the detection of colorectal cancer at the selected cut-off value are 65% and 97% respectively. All persons with a positive screening test will be offered further assessment in hospital.

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The follow-up examination consists of an internal surveillance examination of the colon, known as colonoscopy. In preparation for the colonoscopy, the person is asked to drink a strong liquid laxative at home. The aim is to clean and empty the colon, so the stool is watery and almost clear. In most cases, pain and sleep medication (fentanyl, midazolam) is administered before the colonoscopy. The examination takes about 20 minutes. If possible, polyps are directly removed. If this is not possible, a small piece of tissue is removed (biopsy) and examined by the pathologist.

A. Provide two arguments why the Minister of Health, Welfare and Sports selected iFOBT as the preferred screening test. (4 pt)

B. Based on the information provided above, how many persons on a yearly basis will be referred for further assessment in hospital on the basis of the iFOBT test? (4 pt)

C. Explain that the introduction of colorectal cancer screening can both increase and decrease the incidence of colorectal cancer. (4 pt)

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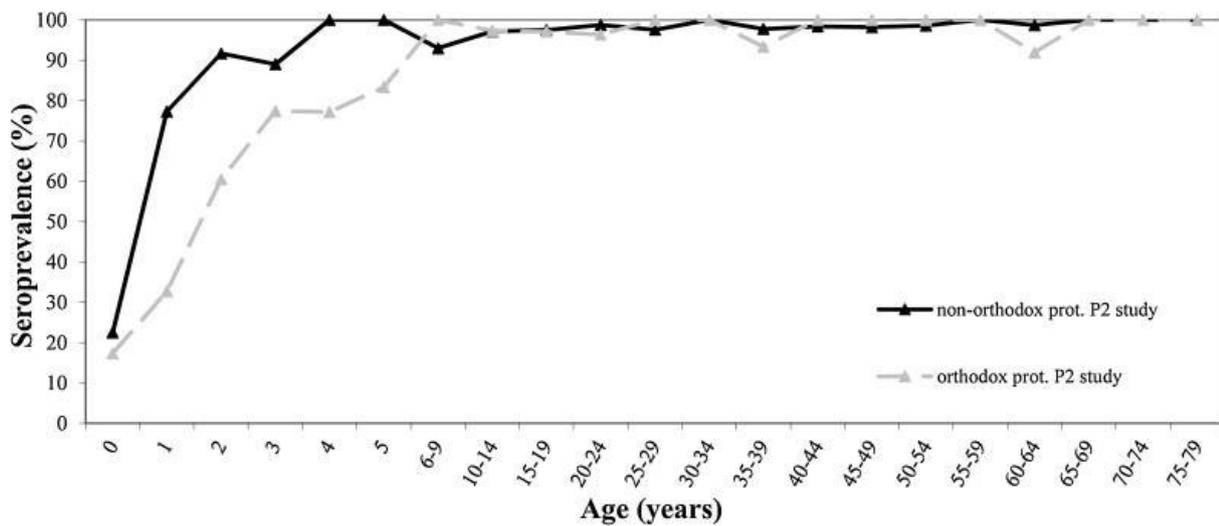
- D. Several years ago, a general practitioner took the initiative to offer his 1300 patients a blood-based test (FOBT) for colorectal cancer. This was before the Minster had decided to implement a screening programme in the Netherlands. The general practitioner argued: "Yearly, 4500 persons in the Netherlands die of colorectal cancer. So early detection is of paramount importance." Do you agree with the general practitioner? Provide two arguments for or against. (5 pt)

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## Vaccination

### Question 2 – Rubella (10 pt)

Although rubella disease is typically mild, infection during pregnancy can result in miscarriage or congenital rubella syndrome (CRS). The rubella vaccine was introduced in the Netherlands in 1974, initially only for girls. Since 1987 all children are vaccinated against rubella. The graph below shows the weighted age-specific seroprevalence for rubella – the percentage of persons in a population with detectable antibodies specific to rubella virus – in Orthodox Protestant individuals (OPIs) and non-OPIs in an area with low vaccination coverage.



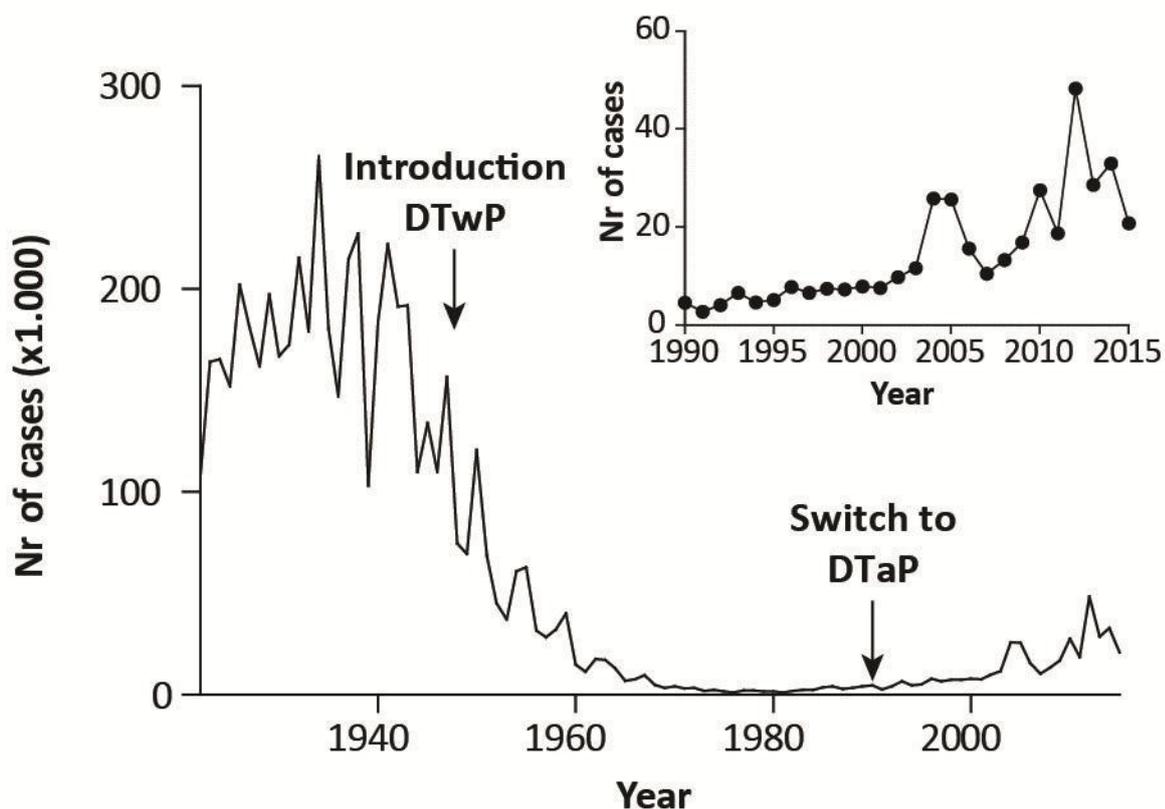
- A. Explain the differences and/or similarities in seroprevalence at the age of 2 and 10 years of age between the OPI and the non-OPI group. (5 pt)

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- B. Not reaching a critical vaccination threshold for infant rubella vaccination can have dramatic consequences. In this context, describe the changes in protection levels in women of child-bearing age over time (short term and long term) following implementation of a rubella childhood immunization program. (5 pt)

### Question 3 – Pertussis (14 pt)

Both the whole-cell (wP) and the acellular pertussis vaccine (aP) provide protection against disease. The graph below shows the cases of pertussis in the US in the prevaccine area as well as after the introduction of the whole cell pertussis vaccine (DTwP) in the 1940s and the later switch to the acellular pertussis vaccine variant (DTaP). In the USA, the vast majority of children is vaccinated against pertussis.



- A. For both the wP and the aP vaccine, describe the two major advantages (benefits) and two disadvantages (risks) in national immunization programs. (4 pt)

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- B. Various vaccine companies are actively developing new safe and improved pertussis vaccines. What are the two major objectives of a Phase III clinical trial with this vaccine? (4 pt)
- C. Explain two major difficulties regarding the design of a Phase III trial with such a vaccine in a country with a high vaccination coverage. (4 pt)
- D. Describe one way how a correlate of protection could facilitate this process. (2 pt)

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**Question 4 – Mechanisms of protection (6 pt)**

Following injection of a vaccine, there are several essential steps in the immune response that ultimately result in the generation of antibodies and clinical protection. Describe the three cell types that are essential for antibody production and describe for each of these cell types in 1-2 sentences their function in this process.

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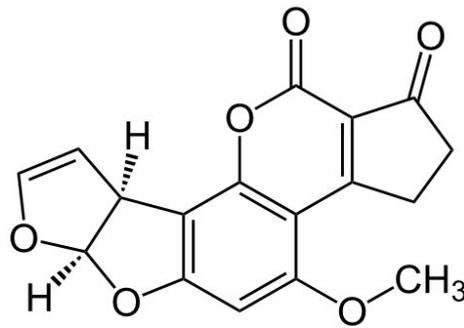
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## Risk assessment

### Question 5 – Aflatoxine (15 pt)

Aflatoxin B1 is produced by *Aspergillus flavus* and is a very potent carcinogen implicated in hepatocellular carcinoma in humans. It is a common contaminant in a variety of foods including peanuts, corn, and other grains.



Aflatoxin B1

*In 2009 the European Food Safety Authority (EFSA) concluded that public health would not be adversely affected by allowing the levels for total aflatoxins from 4 µg/kg to 10 µg/kg for all nuts. An increase in average total dietary exposure of approximately 1 % was estimated. This was based on a better characterization of the variability in the exposure assessment of aflatoxin.*

- A. What is the difference between variability and uncertainty in risk assessment? (3 points)

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B. Name one variability factor and one uncertainty factor that could play a role in the exposure assessment of aflatoxin B1. (3 pt)

C. Preliminary toxicokinetic data of aflatoxin were obtained in a human volunteer study. After an oral dose of 31 ng an AUC of 7 pg.hr/L was found. What can you conclude about the oral bioavailability of aflatoxin: could it be 100% or was it much less? Motivate your answer. (5 points)

D. Aflatoxin B1 requires metabolic conversion to its 8,9-epoxide in order to cause cancer. Draw the biotransformation product aflatoxin B1-8,9-oxide and explain on a molecular level how this reactive intermediate causes cancer. (4 points)

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### Question 6 – Toxicity of Formaldehyde (18 pt)

Formaldehyde is used for fixation of human remains in anatomy and pathology. Students receive their anatomy training in a room where (parts of) human remains are used that are treated with 3-4 % aqueous solution of formaldehyde in water ('formaline'). This year the University of Utrecht decided that pregnant students are not allowed to participate in the anatomy training any more. This decision was based on a meta-analysis indicating an effect of formaldehyde exposure on the rate of spontaneous abortions (see below).

#### Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review

*Anh Duonga, Craig Steinmaus, Cliona M. McHalea, Charles P. Vaughanc, and Luoping Zhanga*  
School of Public Health, University of California, Berkeley, CA 94720; Office of Environmental Health Hazard Assessment, California Environmental Protection Agency; Oakland, CA 94612; Global Health Sciences, University of California, San Francisco, CA 94143

Mutat Res. 2011 November ; 728(3): 118–138

#### Abstract

*Formaldehyde, the recently classified carcinogen and ubiquitous environmental contaminant, has long been suspected of causing adverse reproductive and developmental effects, but previous reviews were inconclusive, due in part, to limitations in the design of many of the human population studies. In the current review, we systematically evaluated evidence of an association between formaldehyde exposure and adverse reproductive and developmental effects, in human populations and in vivo animal studies, in the peer-reviewed literature. The mostly retrospective human studies provided evidence of an association of maternal exposure with adverse reproductive and developmental effects. Further assessment of this association by meta-analysis revealed an increased risk of spontaneous abortion (1.76, 95% CI 1.20– 2.59,  $p=0.002$ ) and of all adverse pregnancy outcomes combined (1.54, 95% CI 1.27– 1.88,  $p<0.001$ ), in formaldehyde exposed women, although differential recall, selection bias, or confounding cannot be ruled out. Evaluation of the animal studies including all routes of exposure, doses and dosing regimens studied, suggested positive associations between formaldehyde exposure and reproductive toxicity, mostly in males. Potential mechanisms underlying formaldehyde-induced reproductive and developmental toxicities, including chromosome and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, toxicogenomic and epigenomic effects (such as DNA methylation), were identified. To clarify these associations, well-designed molecular epidemiologic studies, that include quantitative exposure assessment and diminish confounding factors, should examine both reproductive and developmental outcomes associated with exposure in males and females. Together with mechanistic and animal studies, this will allow us to better understand the systemic effect of formaldehyde exposure.*

The announcement of the University of Utrecht that pregnancy is a reason to expel students from participation in anatomy training is a big step. Especially because there is not yet a 'formaldehyde-free' alternative for this training. Based on the information provided, you are invited to give your expert opinion to the head of the Anatomy Department who is responsible for the anatomy training of students at Radboudumc.

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- A. How do you weigh the evidence and its relevance from human and animal data (as presented in the abstract)? Use ordinal scale for your rating of the weight-of-evidence: e.g. very weak, weak, moderate, strong, very strong. Also express whether or not you consider the evidence presented as *relevant* for the research question on reproductive outcome, use the rating 'high' or 'low'. Note that you have to make a decision concerning evidence from animal data and human data, separately. Motivate your expert opinion (8 pt)

The human data were previously described as inconclusive. However, this systematic review from 2011 supports a possible association of formaldehyde exposure with a relevant reproductive outcome, but there are some uncertainties expressed by the authors (selection bias and confounding). The evidence of human studies is therefore weighed as 'moderate' but the relevance as 'high'.

- B. What uncertainties do you see in the animal data and in the human data? Explain how they have an impact on uncertainty related to the dilemma of the anatomy training. (4 pt)
- C. If the head of the Anatomy Department would like to continue using formaldehyde how could he/she use the precautionary principle to address the problem? Also explain how the precautionary principle could be implemented in practice. (6 points)