Risiken erkennen – Gesundheit schützen

Pesticide exposure and Parkinson's disease: BfR sees association but no causal relationship

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Parkinson's is a slow progressive neurodegenerative disorder which mainly occurs in later life. In the course of this disease the brain cells that produce the messenger substance, dopamine, die off. This important neurotransmitter conducts impulses between the nerves. If insufficient quantities are present, then the typical symptoms of Parkinson's like muscle rigidity, muscle tremor or loss of movement down to akinesia occur. The causes of Idiophatic Parkinson's Syndrome (IPS) are still largely unknown. Besides age-related degenerative changes and genetic factors, discussions mainly focus on environmental and dietary factors. For instance, pesticides could also be a risk factor. On the basis of a comprehensive evaluation of the available literature, the Federal Institute for Risk Assessment (BfR) outlines its opinion below on whether exposure to pesticides could encourage the disease.

The association between Parkinson's disease and pesticide exposure was examined from two angles. Firstly, epidemiological studies were evaluated. Based on a comparison of the incidence of the disease in a group of the population exposed to pesticides and a non-exposed comparison group, conclusions can be drawn about possible associations. Then, the biological mechanisms of action of a few pesticides were analysed in order to answer the question whether they can indeed induce the reactions responsible for the pathogenesis of Parkinson's. To this end, the substances paraquat, maneb and rotenone were examined by way of example. MPTP, a by-product of a heroin substitute which has led to Parkinson-like symptoms in drug addicts, was also examined. The example MPTP has shown that Parkinson's disease can be induced by chemicals. BfR comes to the following conclusions:

The epidemiological studies evaluated point to an association between exposure to pesticides and Parkinson's disease. However, up to now it was not possible to identify either one individual pesticide or a combination of different pesticides as the trigger. Even if individual pesticides may influence dopamine status, a biological plausibility cannot be sufficiently determined in experiments which could explain the onset of Parkinson's disease. Hence a causal relationship between pesticide intake and the onset of the disease in humans cannot be confirmed at the present time.

1 Subject matter of the evaluation

A considerable amount of scientific literature has been published in recent years on the subject of "pesticides and Parkinson's disease". The Federal Institute for Risk Assessment (BfR) drew on this material in its assessment of a possible association. To recapitulate, the Institute considers not only the possible epidemiological evidence but also biological plausibility on the basis of experimental studies. A total of around 250 publications were examined.

2 Results

Like the Medical Research Council Institute for Environment and Health and the National Centre for Environmental Toxicology, both UK (Brown et al., 2006), BfR comes to the following conclusions. There is indeed an association between pesticide exposure and Parkinson's disease. However, the available evidence is not sufficient to reliably confirm a causal relationship. A concrete causal relationship does not, therefore, exist either for an individual pesticide or for a combination of specific pesticides.

3 Reasons

3.1 Introduction

In Europe and North America Parkinson's disease is the second most frequent degenerative disorder of the central nervous system (CNS) with a prevalence of just under 0.1% in the overall population and of slightly more than 1% in the population aged 60 and older. The symptoms typically manifest as the trias: bradykinesia or hypokinesia, rigour and tremor. This is due to a cell loss of transmitter systems in the central nervous system. The main system affected is the dopaminergic nigrostriatal system although the noradrenergic system may also be damaged. Typical Parkinson's symptoms occur from a reduction of the dopaminergic function by around 70-80%, i.e. when most of the nigrostriatal system has already been irreversibly damaged. The course of the disease is chronically progressive.

(Idiopathic) Parkinson's Syndrome (IPS) mainly occurs in later life (onset 60-65 years of age) without any identifiable causes. The cause of the degeneration is still not clear. Many possible risk factors are under discussion. Environmental and dietary factors like for instance exposure to heavy metals, age-related degenerative changes and, more recently, genetic factors are all being discussed. The varying prevalences of Parkinsonism around the world could be seen as evidence of a possible genetic predisposition. There are growing signs that interaction between genetic predisposition and environmental factors plays a role. In the case of Parkinson's disease amongst younger people (prior to age 50) an exclusively genetic basis for the disease can be assumed on the basis of studies involving twins with 100% concordance in the case of identical twins. The current level of knowledge indicates that for most of the forms of Parkinson's syndrome in older people, there may be interaction between environmental factors, genetic factors, certain characteristics of the brain areas affected and age during the disease which means that this is probably a multi-factor event.

In what follows BfR assesses the possible importance of environmental factors. In this context the Institute restricts itself to pesticides and their interaction with other pathogenic factors of nigrostriatal degeneration.

3.2 Epidemiological evidence

3.2.1 Choice of studies

BfR commissioned a meta-analysis of published epidemiological studies in order to examine the epidemiological evidence. Furthermore, data from the so-called "Geoparkinson Study" financed by the European Union involving several countries, were included in the meta-analysis. The study has not yet been published. However, BfR was given the results in advance for this assessment (Seaton et al., 2005)

From this literature search the studies were selected that meet the following criteria. The study

1) contains data on the recording or definition of pesticide exposure and on the diagnosis method and/or the definition of Parkinson's disease.
2) is a cohort or case control study.
3) contains data on the odds ratio or the relative risk, the related confidence interval and the variance of the odds ratio or provides sufficient information in order to calculate the estimator, the confidence interval and the variance of the estimator.
4) was published in German or English.

62 studies in total were identified which examine associations between environmental risk factors and the occurrence of Parkinson's disease. 38 studies fulfilled the inclusion criteria and were included in the analysis. The main reasons for the exclusion of the other studies were the lack of an odds ratios, insufficient information to calculate the odds ratios as well as too low case numbers. No time restriction concerning the publication of the studies was imposed.

3.2.2 Assessment of exposure recording

Pesticide exposure was recorded in different ways in the individual studies. There are differences regarding the definition of exposure, the level of exposure and the time of exposure in relationship to the manifestation of symptoms and the way in which the test persons were interviewed. The test persons were asked about their own use of pesticides or their contact with pesticides at work, in the home or during their free time. The reliability of the answers depends heavily on the test persons' power of recollection and this can influence the study results. In many studies the test persons were only asked about their use and frequency of use of pesticides at work. Other studies, by contrast, recorded their own use at work and in the home or asked the test persons about the use of various products available commercially. In some cases information from the local agricultural office on the consumption of pesticides in the region was also included. Other authors developed a score that covered place of residence, professional activity as well as length of professional activity or defined pesticide exposure as being regular contact for a continuous period of at least six months. In one study there had to have been contact for at least 20 days a year over a period of at least five years. Another study weights the number of years by contact frequency in order to establish a dose-response relationship. In yet another, a distinction was only made between whether there had been exposure for less or more than 20 years. These differences in the recording and assessment of pesticide exposure are partly responsible for the inconsistency of the study results. This might be one explanation for the major degree of heterogeneity in the study results and questions whether a uniform approach can be adopted for all the studies.

3.2.3 The role of confounding

In order to take account of possible confounders in a meta-regression evaluation, during the data extraction it was examined whether the respective studies had been adjusted, for instance, for "smoking" as smoking is under discussion as a protective factor against Parkinson's. This information was not available for all studies. When doing the meta-regression taking into account heterogeneity, the effect of adjustment for smoking disappeared. The evaluation also took account of whether the corresponding studies had been age adjusted which was generally the case.

Even when all the covariates in the meta-regression were taken into account, there was still a clear heterogeneity between the studies. This can be considered to be residual confounding, i.e. there are covariates which were not taken into account but which could influence the result. For instance, genetic predisposition was only considered in more recent studies for instance by Seaton et al. (2005). This could be one explanation of the residual confounding.

When assessing the role of randomness with the help of specific statistical models, statistically significant associations could be identified with the results of Parkinson's disease when considering pesticide exposure. From this it can be concluded that these associations are not random.

3.2.4. Association or causal relationship

Hill's criteria (1965) were used to assess the results regarding a causal relationship. They make a major contribution to assessing complex associations between disease and possible risk factors.

The quantitative analysis was done with statistical methods, stratified according to the substance groups herbicides, insecticides and pesticides. There were contradictory results for herbicides and insecticides. Whereas some of the studies pointed to an association between exposure and Parkinson's disease, other studies pointed to a protective effect. Also when considering a specific substance like paraquat, a consistent association could frequently not be found. In the case of men and women exposed at work and at home, there was also a certain degree of heterogeneity. However, overall a positive association was found between pesticide exposure and Parkinsonism. Overall, there is therefore a relatively consistent association in the global consideration of pesticides.

Another Hill criterion, the lack of alternative explanations, was used. In the studies there are, however, alternative explanations. Exposure to pesticides is only a potential risk factor. The major degree of heterogeneity between the studies also points to the presence of non-considered factors.

The dose-response relationship was examined using various operationalisations. In this context an association could be shown between the dose and the odds ratio. Although the operationalisations were rough simplifications, a dose-response relationship between exposure and Parkinson's disease can still be assumed.

Not all of Hill's criteria must be met in order to establish a causal relationship. One essential criterion is, however, the presence of a time association. In epidemiology the time association between exposure and the onset of the disease can only be assessed as a rule in cohort studies. In a separate assessment of the results – only four cohort studies were available – no association bearing in mind the heterogeneity observed was established between pesticides and Parkinsonism. A time association between reported exposure and the onset of disease cannot, therefore, be unequivocally shown.

By way of summary it can be said that consideration of the association between pesticide exposure and Parkinsonism reveals both weak (odds ratio 1.3) as well as moderate associations (odds ratio 2.16). A consistently higher odds ratio was observed in particular when considering the private and professional exposure of men and women to non-specific pesticides. This consideration does, however, have the disadvantage that many of the substance groups were classified under one name. If one considers specific associations like for instance insecticides, herbicides in general or more particularly the herbicide paraquat, then the results are inconsistent. The studies analysed show a clear heterogeneity. In some of the studies a protective effect was observed. Hence, only the association between pesticides in general and Parkinsonism can be described as relatively consistent.

3.3 Biological plausibility

3.3.1 Mechanistic approaches

The epidemiological results point to an association between pesticide exposure and Parkinson's disease. In order to underdertake an assessment of a causal relationship, mechanistic approaches to substantiate biological plausibility must be used. More recent explanatory

models for the onset for Parkinson's disease, therefore, endeavour to create an initial experimental basis of understanding. Specific approaches from biochemistry but also from molecular epidemiology should clarify whether the onset of Parkinson's disease is caused solely or at least partly by pesticide exposure and how the interaction between genetic and environmental factors is to be understood. Up to now the biochemical mechanisms of the pathogenesis of Parkinson's disease have not been fully elucidated. The available results can, therefore, only be seen as signs of a possible causal relationship which require further scientific examination.

For biological understanding it is important to know that in some animal models damage to dopaminergic neurons and Parkinson-like symptoms can be induced through the administration of specific substances. In this context it is relevant that specific pesticides contribute under experimental conditions to damage to dopaminergic neurons *in vivo* and *in vitro* and typical Parkinson's symptoms and the histopathology typical for IPS can partially be reproduced. The molecular mechanisms involved here are diverse and intervene in fundamental cellular processes of energy and transmitter metabolism. As some of these mechanisms have since been examined in great depth, they are presented here.

3.3.1.1 Lewy bodies

The most noticeable cellular manifestation of nigrostriatal degeneration in conjunction with IPS and most familiar forms of Parkinson's syndrome are intracellular protein aggregates, so-called Lewy bodies (LB). The main component of LB are pathological polymers of α-synuclein, a presynaptic protein which is to be found in the entire central nervous system. Beside polymers of α-synuclein there are also parts of the ubiquitin-proteasome system (UPS) and heat shock proteins (HSPs) in the LB. UPS is the most important enzyme complex for the repair and degradation of damaged cytoplasmatic proteins. HSPs are a large group of proteins with antioxidative and stabilising functions. They are expressed to a higher degree in conjunction with the elevated occurrence of damaged proteins and support UPS activity. Given their composition and location, LB can be seen as the expression of a disturbed degradation of abnormal proteins.

3.3.1.2 Ubiquitin-proteasome system (UPS)

UPS is of key importance for a series of basic cellular processes, including the modification and degradation of proteins. Disruptions to its functioning seem to be both a result and cause in the process of neuronal degeneration with IPS and other degenerative disorders of the central nervous system. Inhibition of UPS in conjunction with IPS could result on the one hand indirectly from a change to the structure of its substrate, α-synuclein, or on the other from direct damage to its 26S sub-unit through oxidants. Numerous factors with a detrimental effect on the function of UPS in the pathogenesis of Parkinson's syndrome have been identified in recent years including gene mutations and toxic processes. Causes for the failure of UPS include more particularly

- oxidative stress, encouraged by mitochondrial dysfunction and the action of specific substances;
- properties of the tissue affected with elevated intrinsic exposure to oxidative metabolic processes;
- the occurrence of damaged proteins with modified confirmation and aggregation properties caused by oxidative stress and
- gene mutations – above all mutations of the genes for α-synuclein, parkin and UCH-L1 with modified substrate and enzyme properties.

α-synuclein probably plays a key role in the failure of UPS in IPS. Hence, UPS inhibition in the IPS animal model in α-synuclein knock-out mice is for instance far lower.

3.3.1.3 Oxidative/nitrosative stress and inhibition of complex I in the mitochondrium

In pathobiochemistry oxidative stress constitutes another set of factors contributing to nigrostriatal degeneration. It is closely linked to protein aggregation and is caused by an elevated cellular strain from unstable and highly reactive compounds mainly peroxynitrite and the hydroxyl radical. Oxidative stress plays a role in many disorders not just IPS. However the cell population most affected in IPS - dopaminergic neurons of the substantia nigra pars compacta (SNc) - show a higher predisposition and vulnerability to oxidative stress.

Despite considerable research over the last two decades the causes of oxidative stress in IPS have still not been clearly elucidated. The phenomenon can be partially explained by specificities in the energy metabolism of dopaminergic cells and the chemical properties of the neurotransmitter produced by them. Damage to the mitochondrial electron transport chain, elevated concentrations of metals and, more particularly, of transition metals like iron, copper and manganese in the brain areas concerned, the dopamine metabolism and inadequate protective mechanisms are known causes of oxidative stress.

Oxidative stress inhibits various metabolism processes including mitochondrial energy production and protein modification and degradation through UPS. In this context it has been shown that polymerised α-synuclein in LB and in the inclusion bodies of other degenerative disorders of the central nervous system shows characteristic changes in the form of nitrotyrosine which can be generated, for instance, by the action of peroxynitrite. The findings of an oligomerisation of α-synuclein by covalent bindings in the presence of peroxynitrite backs this observation. Another manifestation of oxidative stress is damage to DNA and lipids.

3.3.1.4 Cell death in nigrostriatal degeneration – apoptosis versus necrosis

Cell death linked to dopaminergic degeneration is probably both apoptotic and necrotic. On the one hand there are numerous findings on apoptosis and fewer works on non-apoptotic cell death. On the other hand it should be borne in mind that necrotic cell death can be identified less specifically using markers than apoptosis.

3.3.2 The role of model substances and pesticides in nigrostriatal degeneration

Certain substances, as well as some pesticides, can clearly be directly involved in almost all of the above-mentioned processes of cellular cascade damage in IPS. They may promote conformation changes to α-synuclein and promote fibril formation, inhibit enzyme complexes of the respiratory chain and, in this way, prevent both energy harnessing and also contribute to the elevated production of free radicals, lead to depolarisation of the mitochondrial membrane potential and cause, amongst other things, apoptosis. Redox-active substances like certain pesticides and, above all, metals can lead directly to the formation of radicals and, by extension, to the peroxidation of lipids, DNA and proteins. The mechanisms of action of MPTP/MPP+ and a few pesticides are presented and discussed below.

3.3.2.1 MPTP/MPP+

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) was the first chemical substance for which selective damage to SNc in humans and other species could be demonstrated. MPTP is one example which shows that a chemical can induce Parkinson's syndrome and that IPS

could also be caused by other environmental factors. Furthermore, numerous findings on the biochemistry of nigrostriatal degeneration and important therapy approaches have been obtained from MPTP animal models.

In primates MPTP induces a severe, irreversible Parkinson's syndrome which reproduces all typical symptoms of Parkinson's disease in human beings with the exception of resting tremor. In terms of pathomorphology when administered sporadically MPTP causes a selective nigrostriatal lesion and is, therefore, the golden standard of Parkinson's models. Unless otherwise indicated, the findings presented below come from animal models with the sporadic administration of MPTP. The MPTP model with continuous MPTP application comes closest to the pathomorphology of IPS in humans because only in the case of continuous administration damage to the monaminergic systems beyond the dopaminergic system and the characteristic cellular pathology with protein aggregates in the form of LB are obtained. In the case of systemic administration, MPTP crosses the blood-brain barrier within minutes and is metabolised in the glia and serotonergic neurons by monoaminoxidase B (MAO-B) into MPDP+ and MPP+. MPP+ is then carried by monaminergic transporters to all types of monaminergic neurons. This results in selective toxicity for dopaminergic neurons as, for example, scarcely any toxic effects are observed in adrenergic cells of the adrenal cortex in conjunction with comparatively higher tissue concentrations of MPP+. In the neuron MPP+ can bind to the vesicular monoamine transporter-2, can accumulate in the mitochondria or remain in the cytosol and interact with enzymes there. Accumulation in the mitochondrium with inhibition of complex I of the electron transport chain is probably the most important factor for the cytotoxic effect on dopaminergic neurons. The inhibition of complex I by MPP+ leads to a reduction of ATP production and to an elevated production of free radicals. Both effects could contribute to the degeneration of dopaminergic neurones. What seems to indicate the importance of reduced energy supply in MPTP-induced nigrostriatal degeneration is that reduction of ATP production after administration of MPTP in the striatum and central midbrain is particularly developed and that an increase in ATP production via complex II through administration of a ketone body considerably reduces degeneration in MPTP-treated mice. Also synaptic mitochondria seem to be particularly sensitive to ATP reduction by inhibition of complex I.

A second effect of the complex I blockade by MPP+ is the elevated production of reactive oxygen species (ROS). Here there are reports of production of ROS proportional to the degree of complex I inhibition. What also seems to indicate the importance of elevated ROS production in MPTP-induced nigrostriatal degeneration is that modulations of antioxidative enzyme systems, like manganese superoxide dismutase, influence the scale of MPTP-induced neurotoxicity.

Cell death caused by inhibition of complex I in the MPTP animal model is probably mainly caused by apoptosis. The apoptosis path goes through p53-mediated upregulation of Bax, translocation of Bax to the mitochondrium, mitochondrial release of cytochrome-c and activation of the caspases 9 and 3. Bax clearly plays a key role here as genetically modified mice without expression of Bax are resistant to MPTP toxicity. Furthermore, MPTP leads to the accumulation and nitration of α-synuclein in dopaminergic neurones, a characteristic of nigrostriatal degeneration of IPS in humans. The continuous administration of MPTP also leads to the formation of IPS-typical α-synuclein and ubiquitin-containing inclusion bodies in the dopaminergic and noradrenergic neurons.

By way of summary, MPTP/MPP+ leads by means of selective inhibition of the mitochondrial complex I with reduced ATP and elevated ROS production as well as the accumulation

and aggregation of α-synuclein to the described degeneration of SNc, probably primarily by means of apoptotic cell death.

3.3.2.2 Paraquat

Since the discovery of MPTP the bipyridyl paraquat (PQ) is suspected of being neurotoxic because of its structural similarity to MPP+ discussed here. In the European Union (EU) paraquat is authorised for use as a herbicide. This also applies to another substance in the bipyridyl group – diquat.

The evidence for the neurotoxicity of PQ in experimental studies is clear: when administered systemically *in vivo* PQ leads to nigrostriatal degeneration with selective loss of dopaminergic neurons in the animal model of the mouse. When administered systemically (i.e. intraperitoneal injection) PQ crosses the blood-brain barrier and is relatively evenly distributed in the various brain areas including the cortex, striatum, midbrain and cerebellum although there are far lower PQ concentrations in the brain than in other organs like for instance the lungs, kidneys or heart. PQ uptake in the brain is probably via LAT-1, a transporter of neutral amino acids. One indication is that the parallel administration of other LAT-1 substrates can reduce the PQ concentrations in the brain. A two-fold intraperitoneal injection of 10 mg/kg leads to a loss of around 30% of dopaminergic neurons in the SNc of the mouse. In parallel to the reduction in cell density, a highly significant increase in cells with signs of lipid and protein peroxidation was observed which is understood to be a sign of oxidative stress. In particular the proteins of the damaged cells had the characteristic of nitrotyrosines. This is a sign of oxidative damage by peroxynitrite, which is also found in the LB of human beings in conjunction with α-synucleinopathies in the same way as in IPS. These observations are backed by findings of a PQ-induced conformation change of α-synuclein with acceleration of the α-synuclein fibril formation *in vitro* as well as a PQ-induced upregulation of α-synuclein with dose-related formation of amyloid-like fibrils *in vivo*.

The mechanism of action of PQ is, however, different from that of MPTP/MPP^+. PQ is probably not actively accumulated by mitochondria and does not seem to trigger any specific inhibition of the mitrochondrial complex I. Nevertheless, the findings mentioned above do seem to indicate that oxidative stress plays an important role in PQ-induced nigrostriatal degeneration. This assumption is backed by the findings of a protective effect of

- superoxide-dismutase/catalase mimetics *in vitro* and *in vivo*,
- ferritin overexpression and
- coenzyme Q10.

Regarding the mechanisms of action of PQ-dependent ROS production, the following findings are available: a number of studies have shown that PQ can lead to the production of various ROS in the presence of oxygen through redox cycling. Particularly at elevated concentrations of H_2O_2, which are possible for instance in conjunction with dopamine metabolism or in the presence of redox-active iron, PQ also leads to the formation of the hydroxyl radical. In this context it could be shown that the cytotoxic effect of PQ can be considerably reduced by inhibiting dopamine intake or nitroxide synthase (NOS). These observations could indicate that H_2O_2 occurring in conjunction with dopamine metabolism enhances the PQ-mediated production of ROS and, more particularly, the production of the hydroxyl radical. The PQ-induced cell death is, as in the case of the MPTP/MPP+ discussed previously, probably apoptotic. PQ led by means of stress-activated protein kinases (SAPKs or JNKs) to caspase 3 activation.

By way of summary when administered systemically under experimental conditions PQ damages the nigrostriatal dopaminergic system. This effect of PQ probably results mainly from the production of ROS through redox cycling of PQ whereby this effect in dopaminergic neurons could be particularly strong owing to dopamine metabolites as well as redox-active iron compared to other neurons.

3.3.2.3 Maneb

The fungicide maneb (Mn-EBDC), authorised in Europe, belongs to the group of dithiocarbamates (DTCs). With regard to toxicity for the nigrostriatal dopaminergic system the following are described as mechanisms of action of Mn-EBDC: inhibition of several complexes of the respiratory chain and more particularly complex III, an inhibition of proteasome activity and modification of transmitter status in the synaptosom with an increased concentration of dopamine, particularly with parallel exposure to other toxins. Numerous studies have shown that Mn-EBDC in the animal model has a synergistic effect with other toxins for instance with PQ and MPTP. These findings indicate that a number of various DTCs could change the kinetics of other toxins and the metabolism of endogenous substances resulting in higher neurotoxicity. The manganese contained in Mn-EBDC could also lead to Parkinson's syndrome following a possible dissociation of Mn-EBDC into manganese and EBDC. The importance of manganese in the pathogenesis of IPS is, however, questionable.

3.3.2.4 Rotenone

The isoflavonoid rotenone has not been authorised as a plant protection product in the Federal Republic of Germany since 1987. No information is available to BfR about its use in the non-agricultural sector. It is said to be recommended for organic farming (FIBL, 2003). Rotenone-containing pesticides may still be authorised in other EU Member States. According to the information available to BfR this is the case in Austria and Switzerland. It is also said to be used as an insecticide in the USA.

For some years now rotenone has been used in Parkinson's research as typical Parkinson's symptoms as well as histopathological characteristics are reproducible in animal models with rotenone. Furthermore, given its characteristic of being a high-affinity inhibitor of complex I of the respiratory chain, rotenone is used as a mitochondrial toxin in cell cultures. Given its lipophile properties, rotenone easily penetrates the blood-brain barrier following subcutaneous and intraperitonal administration and leads to an inhibition of the mitochondrial complex I in the entire central nervous system. A few days after treatment of rats with rotenone intravenously over a period of several weeks at a dose of 2-3 mg per kg body weight and day, selective degeneration of dopaminergic neurons of the substantia nigra and of the striatum were observed. LB-like fibrillar cytoplasmatic inclusions were found in the neurons of the substantia nigra and the animals were observed to suffer movement disorders like hypokinesia, unsteady movements and bent posture. Some manifested rigidity as well as trembling of the paws which was interpreted as regressive resting tremor. The observed changes, in particular the degeneration of nigrostriatal dopaminergic neurons, movement disorders and cytoplasmic inclusions, are similar to those observed in Parkinson patients.

More recent findings would seem to question the selective degeneration of dopaminergic neurons and IPS-typical composition of the inclusion bodies *in vivo* described for rotenone in earlier works as the inhibition of the mitochondrial complex I is pronounced in the entire central nervous system. It is suspected that the mainly nigrostriatal degeneration can be attributed rather to increased vulnerability of dopaminergic neurons. The effect of rotenone is probably based on a synergistic effect of cytotoxic mechanisms. They include more particu-

larly the high-affinity inhibition of the mitochondrial complex I with a resulting reduction of ATP production and elevated ROS production and direct interaction between rotenone and α-synuclein, the accumulation of α-synuclein and accelerated fibril formation. As a consequence of the inhibition of complex I, the reduction in the ATP production and an elevated generation of ROS probably contribute to cell death of dopaminergic neurons. Attention is drawn to the importance of the glia concerning the elevated production of ROS. The formation of α-synuclein-containing inclusion bodies induced by rotenone is mirrored in *in vitro* studies in which an accumulation of α-synuclein was described in conjunction with an elevated cytoplasmic ubiquitin concentration. The formation of α-synuclein fibrils and inclusion bodies is promoted on the one hand by ROS. On the other hand it has been shown in *in vitro* studies that rotenone interacts directly with α-synuclein and could lead in this way to a conformation change of α-synuclein with accelerated fibril formation. As already mentioned above, it is now contentious whether the cytopathology induced by rotenone is in fact typical for IPS (i.e. typical for synucleopathy) and should perhaps rather be seen as a typical tauopathy. Cell death in conjunction with rotenone exposure manifests characteristics of apoptosis. Here it has been shown that the rotenone-induced elevation of the H_2O_2 concentration leads to the breakdown of the mitochondrial membrane and, in this way, to the release of cytochrome-c and an activation of caspase 3.

4 Pesticide exposure and Parkinson's syndrome – Summary evaluation

The results of the meta-analysis of 38 epidemiological studies point to a relatively consistent association between pesticide exposure and the possibility of developing Parkinson's disease. The identification of a specific pesticide or a combination of pesticides does not, however, result from the epidemiological studies. Nor can any time association be derived between exposure and the onset of the disease. Only a roughly simplified dose-response relationship can be identified. The data available are not sufficient in order to observe a causal relationship between pesticide exposure and the onset of Parkinson's disease.

In addition to these results from the epidemiological studies it can be shown that the typical symptoms of IPS and the underlying tissue changes in the central nervous system can be reproduced through the application of some pesticides in experimental and mechanistic studies. By means of repeated or longer parenteral application of some common pesticides a dopamin deficiency can be induced in various animal experiments. The temporal and local dynamics of the biochemical mechanisms and the resulting behaviour have only been understood to a limited degree up to now.

In the past MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) – a by-product of the synthesis of a heroin substitute – led to Parkinsonism in drug addicts and attracted the attention of research into the causes of Parkinson to the hypothesis of the involvement of toxic factors in the onset of the disease. As MPTP has a similar structure to the herbicide paraquat, this substance in particular is under discussion as a risk factor for Parkinson's disease. There are also descriptions of Parkinson-like changes in an artificial model involving the application of rotenone. Only limited data are available for other pesticides.

By way of summary, the animal experiments and mechanistic studies indicate a partially selective neurotoxicity of specific pesticides for nigrostriatal dopaminergic neurons. In particular for rotenone and paraquat there are *in vivo* and *in vitro* results which prove their neurotoxicity. The extent to which these findings can be applied to humans has yet to be clarified as the results are from animal models. There are certain indications from these studies on biological plausibility that specific pesticides induce symptoms and can cause corresponding histopathological changes. However, these studies do not suffice in order to understand the patho-

genesis, a difficulty which is understandable against the backdrop of the unknown factors contributing to the onset of the disease. This subject matter requires further mechanistic, animal experiments and epidemiological studies perhaps in conjunction with genetic linkage analyses as it is possible that a genetic predisposition, e.g. along the lines of reduced detoxification capacity, could lead to increased environmental vulnerability (Gasser, 2005).

5 References

5.1 Epidemiology

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