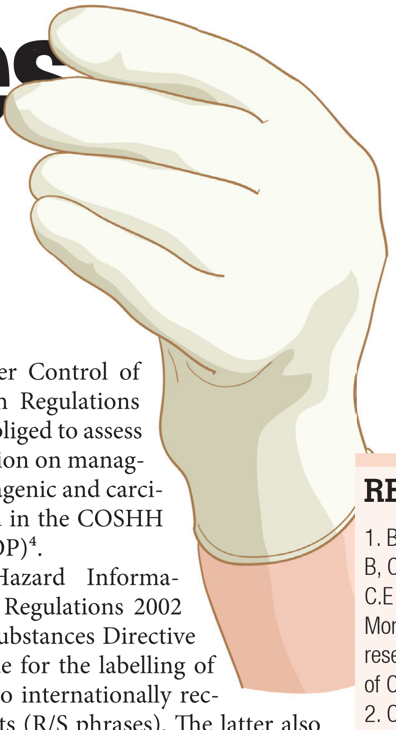
Getting a handle on the right gloves

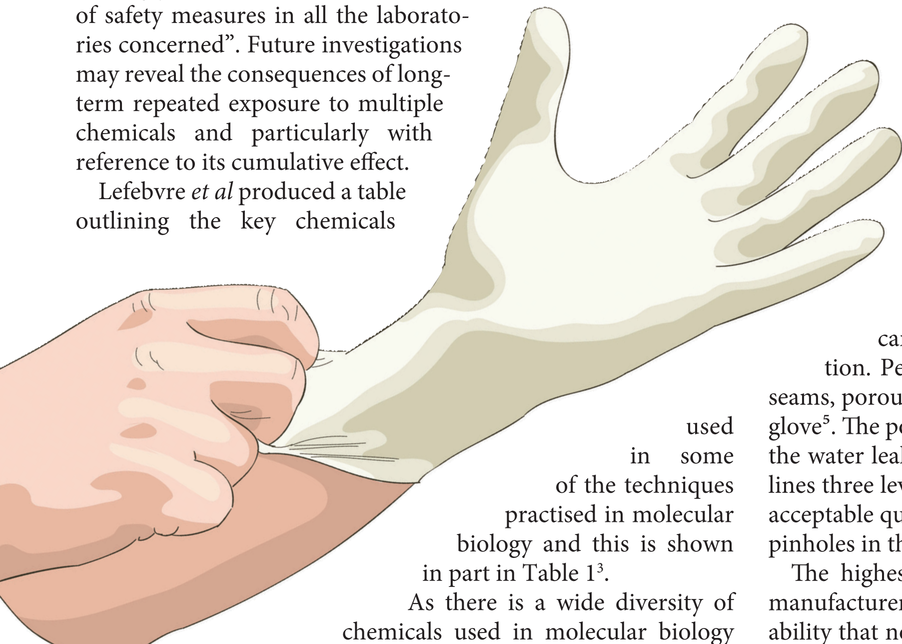
Nick Gardner explores hand protection and chemical hazards in molecular biology



Molecular biology by its very nature deals with biology at the smallest level. Coupled with this, is its relatively recent past and its pioneering ethos. Collectively these factors may have contributed to a subdued level of awareness regarding the hazards facing molecular biologists. This would appear to be the case with hand protection and chemical hazards. Molecular biologists routinely refer to gloves being used for process protection and scarcely mention the need for personal protection from chemical splashes. This seems strange given that powerful genotoxicants such as ethidium bromide and acrylamide are widely used. The purpose of this article is therefore to shed some light on this area and to offer some thoughts on possible solutions for hand protection.

As a nascent science, it is sometimes difficult to get a clear picture on the health risks for molecular biologists[1]. Brown *et al* reported that "there is no evidence of any overall increased risk of mortality in biological research laboratory workers. However, the power of the analysis is limited by the young age of many cohort members and short duration of follow-up." The epidemiologic investigation undertaken by Cordier *et al*[2] at the Pasteur Institute concluded that "work in biomedical research might well involve an increased risk of certain types of cancer. Furthermore whilst it was not possible to demonstrate a causal relationship between molecular biology techniques where potent genotoxic chemicals are used and cases of cancer, the authors "strongly recommend the enforcement of safety measures in all the laboratories concerned". Future investigations may reveal the consequences of long-term repeated exposure to multiple chemicals and particularly with reference to its cumulative effect.

Lefebvre *et al* produced a table outlining the key chemicals used in some of the techniques practised in molecular biology and this is shown in part in Table 1[3].



As there is a wide diversity of chemicals used in molecular biology and the concentrations may vary, it is difficult in an article of this nature to give a comprehensive review. However the chemicals are certainly some of the most commonly encountered, as demonstrated by the frequency with which they are used for different techniques. Lefebvre *et al* also noted that the situation was further complicated by the absence of standardised protocols, whilst individual behaviour was an important factor for determining risk of chemical exposure.

The hazardous nature of many of the chemicals used in molecular biology means that under Control of Substances Hazardous to Health Regulations 2002 (COSHH), employers are obliged to assess the risks. More detailed information on managing the risks associated with mutagenic and carcinogenic substances can be found in the COSHH Approved Code of Practice (ACOP)[4].

According to Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 (CHIP) [Under the Dangerous substances Directive (67/548/EEC)], provision is made for the labelling of hazardous chemicals according to internationally recognised risk and safety statements (R/S phrases). The latter also have a corresponding number. In Table 2, the number and R/S phrase has been applied to highlight the hazardousness of each of the chemicals.

Chemical permeation relates to the process by which a chemical flows through the glove at the molecular level[5]. A breakthrough takes place when the chemical is detected on the other side of the glove and this is shown as the breakthrough time. The standard method for assessing the chemical resistance properties of gloves is EN374-3:2003[6]. Whilst this is a total immersion test and not likely to reflect the laboratory experience, it does enable those engaged in risk assessments to compare the chemical resistance properties of different gloves.

The results highlight the importance of thickness in providing protection. However much of the work of molecular biologists requires a high level of dexterity and tactility. Selection of thin gloves will give users the comfort they desire, but this may come at a price in terms of significantly reduced protection to chemical permeation. As an example your standard gloves will provide minimal protection to chloroform and methanol and yet these are the gloves that are routinely used when exposed to these substances.

Apart from chemical permeation, a chemical can come into contact with your skin through penetration. Penetration refers to the flow of a chemical through seams, porous materials, pinholes and other imperfections in the glove[5]. The porosity of a disposable glove is typically evaluated by the water leak assay as defined in EN374-2:2003[7].The latter outlines three levels of performance (see Table 3), depending on the acceptable quality level (AQL) or the probability of encountering pinholes in the gloves.

The highest performance level of 3 assumes that the glove manufacturer is supplying gloves where there is a statistical probability that no more than 0.65% can have pinholes. Straightaway we can see that all gloves come with an inherent risk of holes and as such even gloves offering maximum protection to permeation may not offer a complete barrier. This is well illustrated with potent genotoxicants such as ethidium bromide and acrylamide, where the resistance to permeation is in excess of 480 minutes but the risk of penetration remains.

A third area to consider when assessing how well protected you are to chemicals is degradation. The latter relates to "a damaging change in one or more of the physical properties of the protective gloves as a result of exposure to chemical agents"[5]. In the presence ▶

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8. EN374-4: 2013 "Protective gloves against chemicals and micro-organisms – Part 4: Determination of resistance to degradation"

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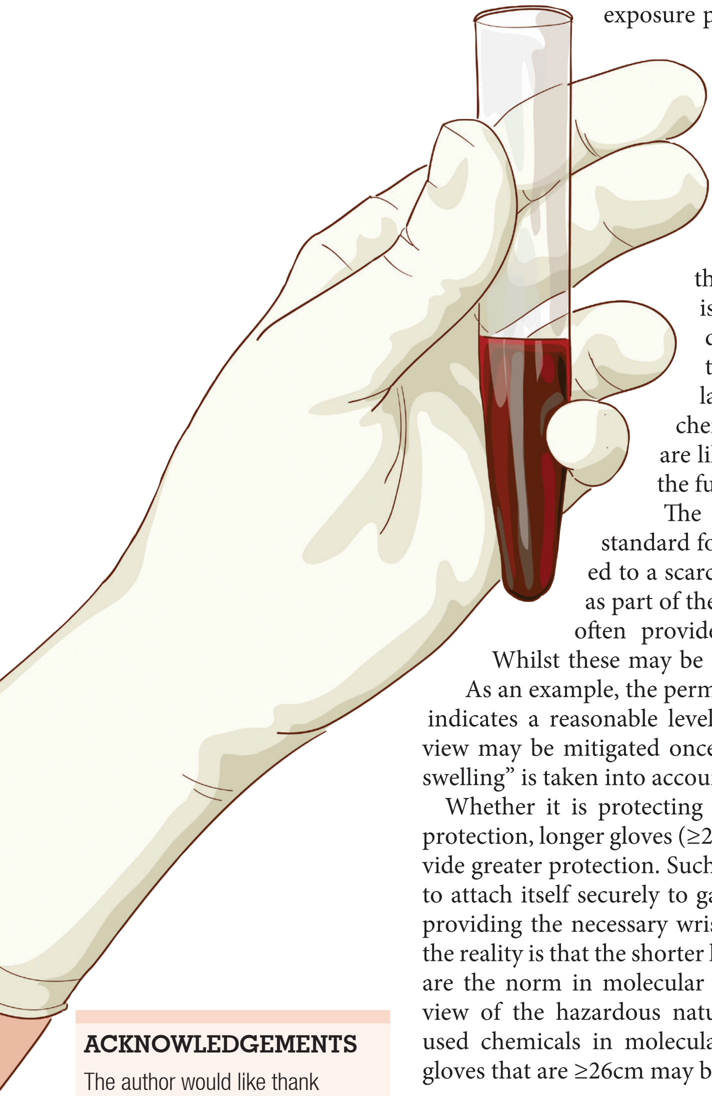


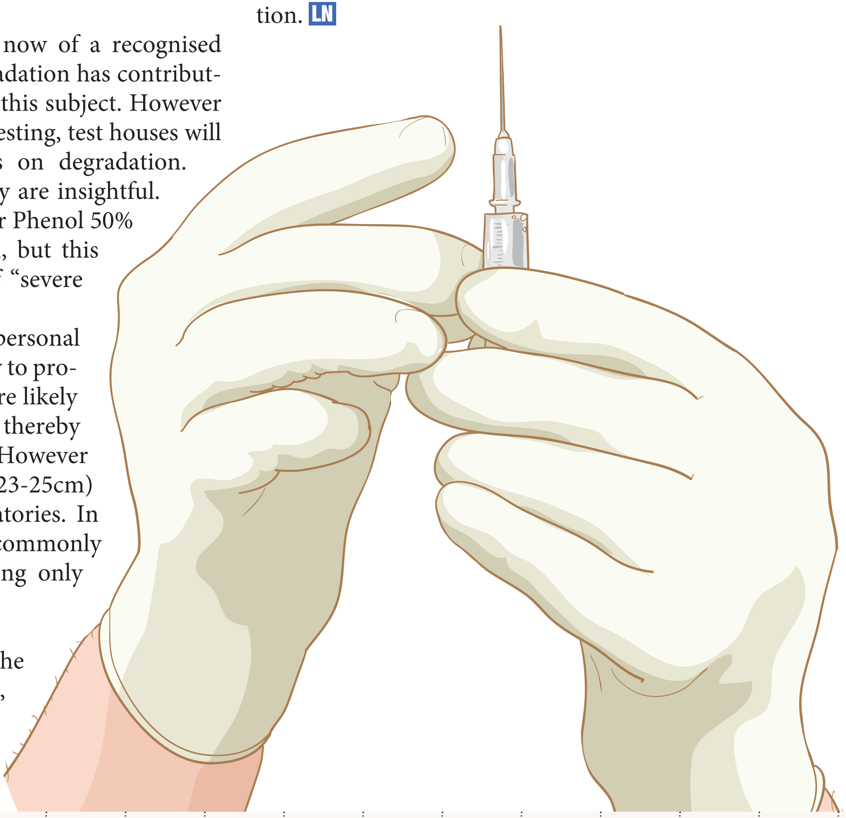
of a chemical, glove materials may become stiff, discoloured or brittle. Likewise they may become softer, weaker and swollen. Resistance to degradation is now measured by a recently published standard EN374-4: 2013[8]. The new degradation test is based on puncture resistance, whilst the glove is in continuous contact with the chemical. At the end of the one hour exposure period, degradation is expressed as a percentage change in puncture resistance (between the unexposed and exposed test specimens). As yet the minimum performance requirements for degradation have not been released, whilst information on how the measurement of degradation will interface with those for penetration and permeation is still lacking. The importance of degradation in reducing the protective properties of gloves is particularly relevant to the more corrosive chemicals. As such degradation ratings are likely to be eagerly sought by users in the future.

The absence until now of a recognised standard for testing degradation has contributed to a scarcity of data on this subject. However as part of the permeation testing, test houses will often provide observations on degradation. Whilst these may be subjective, they are insightful. As an example, the permeation data for Phenol 50% indicates a reasonable level of protection, but this view may be mitigated once the report of "severe swelling" is taken into account.

Whether it is protecting the assay or personal protection, longer gloves (≥26cm) are likely to provide greater protection. Such a glove is more likely to attach itself securely to garment sleeve, thereby providing the necessary wrist protection. However the reality is that the shorter length gloves (23-25cm) are the norm in molecular biology laboratories. In view of the hazardous nature of many commonly used chemicals in molecular biology, using only gloves that are ≥26cm may be preferable.

The above discussion has highlighted the importance of assessing the permeation, penetration and degradation properties of a glove. The variability in resistance to these factors challenges the habitual practice in molecular biology laboratories of having one glove to cover all chemical risks. Often two or three gloves – depending on performance – may be necessary to provide the required level of protection. For some chemicals such as Phenol, it is difficult to see a way forward without substituting this chemical for a less hazardous substance.

Molecular biology is constantly evolving and with this comes different techniques with their associated hazards. Exposure to chemical risks has been highlighted in this article, but equally consideration needs to be given to biological and radioactive hazards. Within the constraints of this short review, it is clear that some of the chemicals used with routine techniques bring considerable risks. Users are faced with a difficult choice – do I favour thinner gloves that give me the dexterity and sensitivity I need in my work or thicker gloves which offer a higher level of protection but reduced comfort. Glove selection may need to extend to considering longer length gloves for better wrist protection and to minimise human-borne contamination of assays. Whilst it may be desirable from a procurement perspective to offer glove wearers minimal choice, the likelihood of one glove meeting all your chemical protection needs is unlikely to be achievable. A differentiated approach to hand protection is probably the best solution. LN



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	DNA Preparation	Total RNA preparation	Separation & Purification of DNA	PCR	Hybridisation	In-situ hybridisation	Cloning	Maxam Gilbert chemical sequencing	Sanger chemical sequencing	Southern blot	Northern blot	Nuclease SI mapping	Run-off & run-on	Footprinting	Gel retardation	Methylation interference	Site-directed mutagenesis	Transfection
Acrylamide			x					x	x			x		x		x	x	
Ethidium bromide			x				x		x	x	x				x		x	
Chloroform	x	x		x	x	x	x		x			x	x	x		x	x	x
Ethanol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Formamide				x	x	x	x	x			x	x		x		x	x	
Isobutanol	x			x	x	x	x	x				x	x	x		x	x	
Isopropanol	x	x					x	x					x	x			x	x
Methanol	x	x					x	x					x	x			x	x
Phenol	x	x		x	x	x	x	x	x			x	x	x	x	x	x	

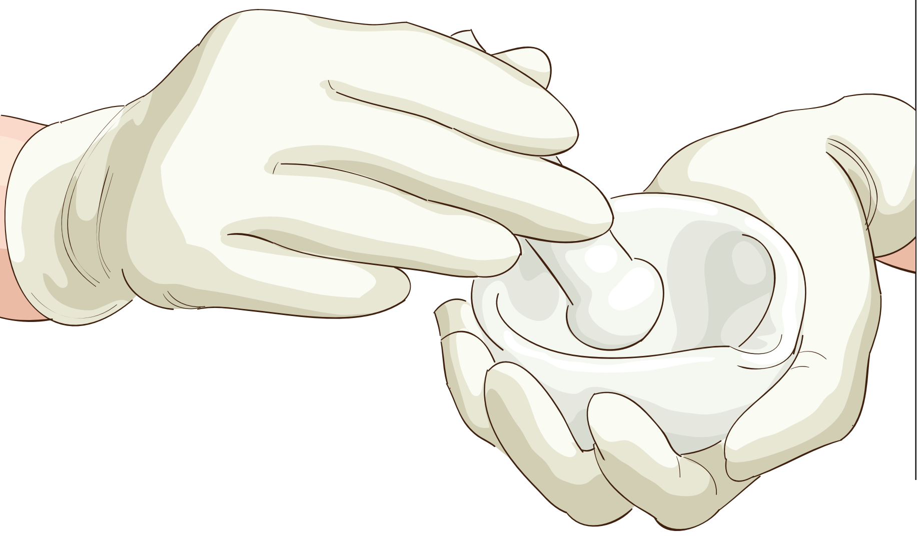
Table 1: Some of most commonly used chemicals and the procedures in which they are used

	CAS NO.	Classification according to Directives 67/548/EEC or 1999/45/EC
Acrylamide	79-06-1	R25: Toxic if swallowed. R48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact and if swallowed R45: May cause cancer. Cat 2 carcinogen. R46: May cause heritable genetic damage. Cat 2 mutagen. R62: Possible risk of impaired fertility. Cat 2 teratogen. R20/21: Harmful by inhalation and in contact with skin. R36/38: irritating to eyes and skin. R43: May cause sensitisation by skin contact. Also some evidence of neurotoxicity[*5] Kjuus et al. (2004).
Ethidium bromide	1239-45-8	R22: Harmful if swallowed. R26: Very toxic by inhalation. R36/37/38: Irritating to eyes, respiratory system and skin. R68: Possible risk of irreversible effects.
Chloroform	67-66-3	R40: Limited evidence of carcinogenic effect. Cat 3 carcinogen. R22: Harmful if swallowed. R48/20/22: Harmful: danger of serious damage to health by pronged exposure through inhalation and if swallowed. R38: Irritating to skin.
Ethanol	64-17-5	R11: Highly flammable
Formamide	75-12-7	R40: Limited evidence of carcinogenic effect. Cat 2 carcinogen. R48/22: Harmful: danger of serious damage to health by pronged exposure if swallowed. R61: May cause harm to the unborn child.
Isobutanol	78-83-1	R10: Flammable R67: Vapours may cause drowsiness and dizziness. R37/38: Irritating to respiratory system and skin R41: Risk of serious damage to eyes
Isopropanol	67-63-0	R11: Highly flammable R36: Irritating to eyes R67: Vapours may cause drowsiness and dizziness
Methanol	67-56-1	R11: Highly flammable R23/25/25: Toxic by inhalation, in contact with skin and if swallowed R39/23/24/25: Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.
Phenol	108-95-2	R23/24/25: Toxic by inhalation, in contact with skin and if swallowed R34: Causes burns R48/20/21/22: Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed R68: Possible risk of irreversible effects

Table 2: Hazardousness of most commonly used chemicals according to R/S phrases

Performance level	Acceptable Quality Level unit	Inspection levels
Level 3	<0,65	G1
Level 2	<1,5	G1
Level 1	<4,0	S4

Table 3: Acceptable quality levels as defined in EN374-2:2003



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compliance comfort protection

ONE RISK, ONE GLOVE !

Red: Stop danger! Chemical risk!
Orange: Be careful! Biological risk!
Green*: Go ahead! Everyday risk!

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